INSECTICIDAL ACTIVITY OF PLANT OILS AGAINST STORED PRODUCT PESTS

BY

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ABSTRACT

The insecticidal activity of plant oils against Callosobruchus maculatus on cowpea, Sitophilus zeamais on wheat and Dermestes maculatus on dried fish has been investigated.

Preliminary experiments showed that all the plant oils tested could reduce the development of progeny in the above species. However, the two groups of plant oils assayed, namely fixed (groundnut, traditional coconut, Palm, traditional palm, corn , sesame, coconut and sunflower) and essential oils (limepeel, orangepeel, tangerine peel, lemon peel, grapefruit peel and mandarinpeel) showed distinctly different mechanisms of action.

When all cowpea grains were treated with fixed oils, oviposition by C.maculatus was not affected although there was considerable subsequent mortality. However, in a two-way choice experiment, C.maculatus showed a strong oviposition preference for grains not treated with fixed oils. X-ray studies showed a similar effect of fixed oils on the oviposition pattern of S.zeamais. In contrast, essential oils reduced oviposition in C.maculatus (mainly by causing rapid adult mortality) but had no residual action on eggs and larvae.

In general, fixed oils applied at rates of up to 14 ml/Kg substrate were shown to have no direct toxicity against active stages of D.maculatus and C.maculatus. Topical application of fixed oils on adults and larvae of D.maculatus confirmed these observations. At rates above 10.5 ml/Kg, freshly applied fixed oils were found to cause some mortality in S.zeamais but this

effect disappeared when treated grains were aged for 14 days before bioassay. Application of fixed oils at up to 14 ml/Kg to grains preinfested with C.maculatus or S.zeamais larvae confirmed that these oils had no appreciable effect against such stages.

Studies with C.maculatus revealed that the main toxic action of fixed oils was against the late embryonic stage, preventing hatching of first instar larvae. However, when fixed oils were dissolved in acetone death usually occurred in the early embryonic stages. The three major constituents of the fixed oils, oleic, linoleic and lauric acids, were shown to have a similar effect to that of the naturally occurring oils; being active only against eggs of C.maculatus.

The biological action of essential oils was shown to depend on a strong fumigant action. Bioassays conducted in air-tight glass chambers showed that all the oils had vapour toxicity towards C.maculatus, S.zeamais and D.maculatus adults. Further studies revealed that all the essential oils tested also had a strong fumigant ovicidal and larvicidal action on all three species which extended to C.maculatus and S.zeamais larvae living inside grains. X-ray studies showed that fumigated C.maculatus larvae within cowpea grains died without further development.

Following gas chromatographic analysis of essential oils, 30 components of these oils were tested against C.maculatus. Several compounds, including the major component of all essential oils - D-limonene were found to be bioactive. These active components and some of their isomers were shown to have varying levels of toxicity and no single component could account for the toxicity

of an essential oil on a proportional basis. Joint action studies established that in artificial mixtures several pure components of essential oils (D-limonene, γ-terpinene, terpinolene, α-terpineol and n-decyl alcohol) had an accumulative action against C.maculatus. Further studies suggested that mixtures of D-limonene and α-terpineol may have an additive action.

Bioassays showed that absorption of essential oil fumes occurred in the presence of grains or strips of dried fish and that this tended to reduce their toxicity. However, studies with C.maculatus showed that this effect did not increase proportionally with increasing amounts of grain.

The fumigant activity of one essential oil (lime peel) and D-limonene with time was determined against C.maculatus and D.maculatus. Mortality was found to occur from 30 min after exposure to lethal concentrations. Observations on treated insects revealed a number of symptoms reminiscent of nervous intoxification. Preliminary investigations into the possible mode of action of essential oils on insects are described.

The potential for plant oils, especially essential oils, as novel alternatives to synthetic insecticides as stored product protectants is discussed.

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DEDICATION

Man's greatest scientific achievements are usually motivated by noble, not so noble or no ideals; my contribution to science, small or great is motivated by the desire for a better tomorrow for the UNDERPRIVILEDGED and UNDERDEVELOPED masses of the world.

This thesis is dedicated to:

the future of my country, Nigeria;

to the genius and care of Celia Oakley, H.H. Bentall and their colleagues at Hammersmith Medical School and Hospital;

and, to the evergreen memory of Papa and Stanley who inspired my academic career.

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Plate 1. Dried fish strips.

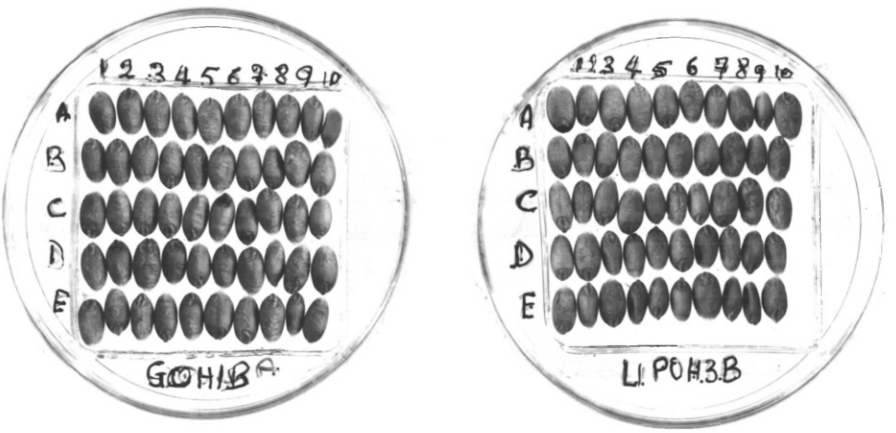


Plate 2. Plastic frames (with Sellotape base) holding wheat grains for X-ray radiography.

GENERAL INTRODUCTION

1.1 Introduction.

The traditional use of biologically active plant products has led to the discovery of a number of important present day biochemicals including pesticides such as pyrethrum and the synthetic pyrethroids (Elliot et al., 1965; McLaren, 1986). A few examples which demonstrate the diversity of origin and range of modes of action of insecticidal plant extracts are given in Table 1.1. Natural products such as nicotine and rotenone were among the first insecticides used. These were superseded by synthetic materials with the development of organochlorines (eg. DDT), organophosphates (eg. parathion) and carbamates (eg. carbofuran) (McLaren, 1986).

Until recently, screening for new pesticides has concentrated on the testing of novel synthetic compounds and chemical analogues of known bioactive compounds. However, there is an increasing trend towards the search for microbial and plant products for several reasons; most importantly perhaps due to environmental and toxicological side effects of many synthetic organic compounds (Loftin, 1945; DeBach, 1951; Newsom, 1967; Don-Pedro, 1976, 1980, 1984), and to often widespread pest resistance to such pesticide groups (Pal, 1974; WHO, 1980; Conway, 1982; Don-Pedro and Adegbite, 1985).

In developed countries, the use of synthetic organic agrochemicals in crop protection is increasingly controlled by strict laws and management systems aimed at minimising attendant

Table 1.1 Diverse examples of plant compounds with insecticidal activity.

Common name	Plant origin	Insect response
Affinin	*Heliopsis longipes*	Paralysis
Ajugarin	*Ajuga remota*	Suspension of feeding
Allylglucosnolate	*Brassica nigra*	Larval inhibition
Angelicin	*Psoralea* sp.	Larval inhibition
Azadirachtin(neem)	*Azadirachta indica*	Supression of feeding
Canavanine	*Canavalia* sp.	Supression of feeding
Citronella	*Andropogon nardus*	Repellance
Ecdysones	Broad range	Hormone mimics
Imperatorin	*Clausena antisata*	Supression of feeding
Nicotine	*Nicotiana tabacum*	Paralysis
Precocenes	*Ageratum houstonianum*	Anti-allatropic
Pyrethrins	*Chrysanthemum cinerariae folium*	Paralysis
Rotenone	Leguminosea	Respiratory inhibition
Ryanodine	*Ryania speciosa*	Stomach poisoning
Xanthotoxin	Apioideae sub-family	Larval death

Adapted from McLaren (1986).

deleterious side effects. However, in most underdeveloped countries, due to the need to preserve and increase agricultural productivity, such procedures are still largely lacking or not enforced. Under the latter conditions, potent synthetic agrochemicals are commonly used as if their benefits always outweigh their negative side effects. In addition, many developing countries cannot afford to import the newer, more expensive and sometimes less-envioronmentally damaging pesticides from abroad. It is worthwhile, therefore, to look for alternative sources of local pesticide production in the regions concerned.

In the above regard, plant components with insecticidal activity could play a major role. Components of natural food products may be the most important sources of such natural insecticides since they are perhaps the least likely to possess harmful side effects to man or his domestic animals. This is the principal reason for the present study into the insecticidal activity of edible plant oils.

1.2 Review of literature.

1.2.1 Insecticidal activity of plant oils

Petroleum (mineral) oil and to a lesser extent vegetable oils are used alone or in combination with synthetic insecticides against a variety of insects, especially to kill dormant eggs (Schoonhoven, 1978). Mixing oils obtained from locally available plants with grains was in fact an ancient Indian and African method of protection against insect attack (Periera, 1983) and most of the reported studies with plant oils have been concerned

with their use against stored grain insect pests.

In Nigeria, merchants rub groundnut oil or other vegetable oils on dried fish for protective or for cosmetic reasons and in some parts of the country orange peels (containing volatile oils) are burned at night to drive off mosquitoes (Don-Pedro, 1985). While in Senegal, the locals are said to rub fish liver oil on dried fish for unspecified reasons (D. Walker and C. Wood, personal communication), although no studies on the use of oils against pests of stored animal products appear in the literature.

The review of Golob and Webley (1980) shows that an increasing number of plant oils have been screened for use in preventing post harvest losses due to insects. A full understanding of the action of such plant oils against stored product insect pests is therefore required.

Singh et al. (1978) showed that groundnut oil applied at 5 ml per Kg of grain protected stored cowpea seeds from attack by the bruchid beetle, Callosobruchus maculatus. Several other vegetable oils have similarly been shown to control various bruchid species, including C.maculatus infesting legumes in laboratory bioassays (Periera, 1983; Su et al., 1972b; Schoonhoven, 1978; Mensina and Renwick, 1983). In similar trials, vegetable oils were found to protect wheat from the granary weevil, Sitophilus granarius (Qi and Burkholder, 1981) and rice from Sitophilus oryzae and Sitophilus zeamais (Zhang and Zho, 1983).

Most workers who have screened vegetable oils against insect infestation of grains have simply demonstrated a reduction in F1

progeny development within highly variable treatment levels and experimental conditions (particularly methods of application). The mechanism of action of these oils against storage insects is also not clear. While practically all reports agree that vegetable oils are contact ovicides, there is less certainty on their activity against other stages in the insects' life cycles.

Singh et al. (1978) reported that groundnut oil applied to cowpea had no effect on mortality or longevity of adult C.maculatus. Similarly Tikku et al. (1981) showed topical application of several vegetable oils, including groundnut and coconut, had no effect on adult mortality of the bruchid, Bruchus chinensis. In contrast, Hill and Schoonhoven (1981) found that palm oil killed adult bruchids while Su et al. (1972a, 1976) demonstrated topical toxicity of citrus peel oils against adult C.maculatus and other beetle pests. Qi and Burkholder (1981) reported that vegetable oils applied to wheat grains were only lethal to adult Sitophilus beetles at high dosage (10 ml per Kg).

The effect of oil treatments on oviposition is also unclear. Most workers have observed a reduction in oviposition of bruchid beetles on oil-treated grains (Mensina and Renwick, 1983; Singh et al., 1978), while others, notably Periera (1983), have reported no significant action.

Apart from the preliminary observation of Mensina and Renwick (1983) on the lack of mortality among larvae that successfully penetrated vegetable oil-treated seeds, there are no detailed reports on larvicidal action of vegetable oils.

Thus, experiments to determine the full range of lethal and sublethal effects of plant oils against insects are lacking. Most reports have assumed that all plant oils act similarly (see Mensina and Renwick, 1983; Singh et al., 1978) and some authors have extended this similarity of action to mineral oils (Mensina and Renwick, 1983). However, such assumptions may be invalid since the physical and chemical properties of various groups of plant and petroleum (mineral) oils differ greatly.

With regard to insect eggs, although the ovicidal action of vegetable oils is not in dispute, the mechanism of action is unclear.

It has been shown that petroleum (mineral) oils depress the rate of oxygen uptake and elimination of toxic metabolites by insect eggs (Smith and Pearce, 1948). However, for oils to kill eggs in this way their volatility must be low enough to allow them to interfere with gaseous exchange for at least 24 h (Fiori et al., 1963) and such an action is therefore unlikely to extend to the highly volatile citrus oils. Singh et al. (1978) proposed that the toxicity of topically-applied vegetable oil to C.maculatus eggs was due to both physical and chemical effects. This view has been supported by Schoonhoven (1978), who showed that vegetable oils of different purity varied in toxicity, and by Gunther and Jepson (1960), who stated that the action of oils was probably more complex than simple physical interference with respiration, as insects deprived of oxygen survived longer than those treated with oils.

1.2.2 Fixed and essential plant oils

1.2.2.1 Terminology.

From the literature on insecticidal plant oils it is apparent that no distinction has been made between types of plant oils on the basis of different mechanisms of bioactivity. In the present work, two groups of plant oils are presented as belonging to two distinct types of insecticidal compounds:

1. Non-volatile plant oils without "essences"; referred to as "fixed oils".
2. Plant oils with volatile "essences" referred to as "essential oils"

1.2.2.2 Fixed oils.

These are oils extracted from certain seeds. They consist primarily of glycerol with three straight-chain fatty acids attached as esters. This large molecule is commonly called a triglyceride and represents about 95% of the weight of most oils from seeds. However, minor components are also present, such as free fatty acids, sterols, sterol esters, phosphatides, glycolipids, hydrocarbons, glyceryl ethers, fat-soluble vitamins, monoglycerides and diglycerides (Sheppard et al., 1978).

The fatty acids normally encountered in oils are composed of 4 to 24 carbon atoms. A number of monounsaturated and polyunsaturated fatty acids may be present. The fatty acid composition of some common fixed oils is given in Table 1.2. Oleic acid is the principal component in these oils with the exception of coconut oil, where lauric acid is the major component.

Table 1.2 Fatty acid composition of vegetable oils.

Fatty acids*	Mean[ab] fatty acid composition ± S.E. (g|100g fat) of selected vegetable oils.					
	Groundnut oil	Coconut oil	Palm oil	Corn oil	Sesame oil	Sunflower oil
6:0	-	0.7±0.9	-	-	-	-
8:0	-	7.5±1.6	-	-	-	-
10:0	-	5.9±0.9	-	-	-	-
12:0[1]	-	43.7±3.1	0.2±0.1	-	0.3±0.1	-
14:0	0.1±0.1	16.4±1.7	1.1±0.4	-	0.1±0.1	-
16:0	9.5±0.9	8.2±1.8	42.0±4.8	10.7±0.8	9.4±2.9	6.3±1.3
18:0	2.3±0.7	3.0±1.8	4.3±1.5	1.7±0.2	4.8±1.6	3.9±0.8
20:0	1.4±0.7	0.9±0.2	0.3±0.1	0.3±0.2	0.6±0.4	0.7±0.8
Total saturated	13.3	86.3	47.9	12.7	15.2	10.9
16:1	-	0.4±0.3	0.5±0.5	0.1±0.1	0.3±0.2	0.2
18:1[2]	45.6±6.0	5.7±1.7	37.9±6.9	24.6±1.7	39.1±3.5	34.0±8.9
18:2[3]	31.0±5.9	1.8±0.9	9.0±23.	57.3±2.5	40.0±4.1	49.4±8.0
18:3	-	-	0.3±0.2	0.8±0.3	0.5±0.3	0.3±0.2
20:1	1.2±0.4	-	-	-	0.2±0.1	-
22:1	-	-	-	-	0.4±0.2	-
Total unsaturated	77.8	7.9	47.7	82.8	80.5	83.9

[a] Minor fatty acids of less than 0.1g not included

[b] Mean and standard error of 15-42 analysis.

* Types of fatty acids (number of carbon atoms: number of double bonds).

After Sheppard et al. (1978).

1 = Lauric acid; 2 = Oleic acid; 3 = Linoleic acid.

At present, the main economic importance of fixed oils lies in their use for cooking and baking, and for soap making. Oils together with fats (oils that are solid at room temperatures) provide the most concentrated source of energy in the human diet, about 9 Kcal/g (Sheppard et al., 1978). These oils are readily available in many tropical countries as the plants from which they are extracted mainly grow in this region.

Most work on the insecticidal activity of plant oils has been carried out with fixed oils (referred to as vegetable oils by most earlier authors). However, very little work appears to have been done on the efficacy of their component fatty acids against insects, or their eggs. The only report in the literature is by Hill and Schoonhoven (1981) who showed that complex triglycerides were the active components of fractionated palm oil and cotton seed oil against bruchid stored product pests.

1.2.2.3 Essential oils.

Extracts from the epicarp of citrus fruit peels which contain oil sacs yield essential oils. The term "essential oil" in general extends to other plant oils with essences (aroma) and they can be extracted from a wide range of non-citrus plants (Guenther, 1961; Lawrence, 1977, 1978).

Citrus essential oils in general contain mainly terpene hydrocarbons and oxy-containing compounds including aldehydes, esters and alcohols. Mchale (1980) showed that filtered lime oil consisted of the following major fractions: monoterpenes, 76.6%; sesquiterpenes 3.8%; oxy-containing compounds 18.1%. Each of these fractions is composed of a wide range of compounds. The

review of Shaw (1979) gives an excellent compilation of individual components (over 100) and their quantitative presence in citrus oils (sweet orange, grapefruit, mandarin, lemon, distilled and cold pressed lime, bitter orange and bergamot).

Citrus species are grown from the French Riviera (45° North) to Auckland, New Zealand (40° South). The most important regions for commercial cultivation of citrus are North America, the Mediterranean, East Asia, Brazil, Argentina, South Africa and Australia (Anon., 1973).

Essential oils are used in the flavouring of all kinds of beverages, confectionaries and baked goods (Swisher and Pritchett, 1969). They are also used in perfumes, cosmetics, and for the scenting of soaps. The antimicrobial or antibacterial action of some citrus oils have been reported by Subba et al. (1967) and Dabbah et al. (1970).

While citrus peels (oils) produce valuable revenue for some tropical countries such as Brazil, Mexico and the Gambia, in others, like Nigeria, the peels are lost as waste (Don-Pedro, 1985). The insecticidal properties of these citrus peel oils may therefore provide an opportunity for economically exploiting such plant products all over the tropics. Don-Pedro (1985) demonstrated that sun-dried citrus peel powder was toxic to C.maculatus and to a lesser extent the hide beetle, Dermestes maculatus. However, the large amounts of peel material required to give adequate control of such insects makes the simple approach of using peel powder as a protectant too cumbersome (Don-Pedro, 1985). It may, therefore, be more realistic to

exploit the insecticidal properties of the peel extract.

While the use of fixed oils to protect grains from insects dates back to ancient times, few attempts have been made to use citrus oils to control insects and these are comparatively recent (Back and Pemberton, 1915; Jacobson, 1958; Lichtenstein, 1966). A computer literature search carried out in the present study found only 20 such reports between 1970 and 1986. Nothing is apparently known about the mode of action of citrus essential oils on insects.

Back and Pemberton (1915) reported that material in the "oil cells" of citrus peel was toxic to eggs and larvae of the Mediterranean fruit fly, Ceratis capitata. More recently, workers have shown the toxicity of citrus peel oils to eggs and larvae of a wide range of tephritid fruit flies (Greany et al., 1983). This observation was given as the factor affording citrus fruits considerable resistance to fruit fly infestation.

Su et al. (1972a), who have perhaps drawn most attention to the insecticidal properties of citrus oils in recent years, have demonstrated topical toxicity for oils from 8 types of fruits against bruchids and other beetle pests. They have also shown that cowpeas could be protected from C.maculatus by the use of citrus oils in laboratory bioassays (Su et al., 1972b). In addition, Abassy et al. (1979) found that fresh residues of citrus fruits and orange peel oils were toxic to the grain weevil

S.granarius and the flour beetle Tribolium castaneum. The latter also showed that citrus oils could synergise the action of conventional synthetic insecticides.

Most of the reports on the insecticidal properties of citrus oils have emphasised their contact toxicity, as shown by topical applications, dipping and pre-oviposition grain applications. However, Taylor and Vickery (1974) did suggest that such oils could also have some fumigant toxicity. More recently, Sheppard (1984) has demonstrated vapour toxicity of materials exuded from scarified orange peels against house flies.

The toxicity of citrus oils to insects has been traced to some of their component chemicals. For example, Taylor and Vickery (1974) found that limonene and some lesser components showed contact toxicity to C.maculatus and house flies in dry film filter paper tests. While Styer and Greany (1983) showed that citral applied by dipping was more toxic than limonene to eggs of the Caribbean fruit fly. Most of these reports have involved simple tests to demonstrate the bioactivity of oil components, and none have attempted to explain the toxicity of oils on the basis of individual components or of several components acting together.

Harbourn (1977), in his book on ecological biochemistry, has observed that mixtures of several closely related bioactive compounds are often produced by plants and it is likely that synergism occurs.For example, one substance enhancing the effectiveness of a second as an insect deterrent. It would, therefore, be interesting and of potential practical value to explore the joint actions of the terpenoid compounds which

constitute the bulk of secondary metabolites in citrus peel oils.

Essential oils from non-citrus plant sources have also been shown to have insecticidal properties (Bestman et al., 1984; Klingauf et al. 1983; Chavan et al., 1983; Yao and Yang, 1984); the reported activities described including contact, repellent and fumigant activity against insects.

1.2.3 Food storage losses

1.2.3.1 Pest problems.

Insect pests cause damage to agricultural crops both in the field and in storage. Prevention of losses in storage are often the more important because they are final without the compensatory mechanisms that may occur with growing plants. Most of the economically important legumes, cereals and vegetable crops are harvested over a very restricted period and in order to ensure supply all year round, a high proportion of the world's agricultural and horticultural produce has to be stored. Without proper storage and preservation methods, considerable losses may occur due to attack by insect pests, rodents, mites, and by bacterial and fungal infections, or to the development of physiological seed disorders including the premature breaking of dormancy.

Pest induced damage includes loss in weight, quality (contamination) and nutritional value as well as loss in goodwill in trading. Most arthropod pests of stored produce belong to the coleopteran and lepidopteran insect orders, and to astigmatan mites; members of the dictyoptera, thysanoptera, diptera and

formicidae are of lesser importance. Other arthropod groups, especially parasitic hymenoptera, and prostigmaton and gamasine mites are seen as potentially beneficial, as biological control agents.

1.2.3.2 Cowpea and cereals.

Cowpea (Vignia unguiculata Walp.) is amongst the principal sources of vegetable protein in the tropics. In Nigeria, this important crop is attacked by several insect pests in storage but predominantly by the bruchid beetle C.maculatus (Agboola, 1982). Bruchid insect species occur as pests of legumes in most of Africa, Asia and South America (Giga and Smith, 1983).

Cereal production is important all over the world. In the U.K., cereal crops (principally wheat and barley) are the most valuable agricultural crops accounting for 16.5% of agricultural production (Wilkin, 1985). The most important insect pests of stored cereals in the U.K. include the following beetles, Oryzaephilus surinamensis, Sitophilus oryzae, S.granarius and S.zeamais (Wilson, 1983). In the tropics, including Nigeria, the most important cereals are sorghum, maize, millet and rice. Wheat is becoming more important in certain areas such as the Lake Chad basin in Nigeria. Tropical cereals in storage are attacked by several insects, notably members of the following genera: Sitophilus; Tribolium and Rhyzopertha (Cornes, 1964). Giles (1964), estimated that about 4% of all sorghum and millet grain was lost to stored product insects in Nigeria. Present day damage estimates which are probably more reliable are between 5-15% by weight (Mrs Mejule, Nigeria Stored Product Research Institute, personal communication).

1.2.3.3 Dried fish.

In West Africa, fish is not necessarily dried for preservation but because of its special flavour. It plays an important role in the traditional diet and pieces of dried fish may be eaten without washing or further cooking as snacks. Hence, attempts to protect dried fish with normal insecticides pose unacceptable risks of poisoning to man. This makes the search for safe, selective protectants for this material most urgent.

Large-scale deterioration in quality and quantity of dried fish results from insect infestations and microbial attack (Cutting, 1961). In West Africa, dried fish is attacked by several insect species, but the most important is D.maculatus followed by the related beetle, Necrobia rufipes (Osuji, 1975). Osuji (1973) estimated that over 4 million naira (3.4 million £ UK at the existing exchange rate) was lost annually to D.maculatus damage to fish transported between the Lake Chad region and southern Nigeria alone.

1.3 Objectives of the present study

1. To compare the insecticidal activities of fixed and essential oils with a view to establishing possible differences in their mechanisms of action against insects.
2. To discover the action of fixed oils against stored produce pests by establishing (a) their mode of action against insect eggs, and (b) which other stages in the life cycle are affected.

3. To investigate the mechanism of action of citrus essential oils against adult and juvenile stages of insects in order to establish the best method of using such oils in storage systems.
4. To investigate the toxicity of citrus oil components alone and as simple mixtures against test insects and to carry out preliminary investigations into the mode of action of citrus oils.

GENERAL MATERIALS AND METHODS

2.1.1 Test Compounds

In this study, industrially extracted oils were obtained from local supermarkets or manufacturing companies in the U.K. and Nigeria (Table 1). This was for two main reasons: 1) If plant oils or other oils are to play a role in large-scale crop protection, the oils or their fractions will have to come from existing industrial processes of extraction. 2) The current study required large quantities of material which could not be extracted in the laboratory due to limitations of raw materials, equipment and time. However, in order to test if the purity of oils was related to their biological activity, some crude oil extracts from traditional manufacturers in rural Nigeria were also used. A total of 14 plant oils and one animal oil were examined (Table 1).

The fixed oils were stored at 28° C, since at lower temperatures most of them solidified. Essential oils of citrus origin were stored at room temperature (ca 20° C).

2.1.2 Test Insects

The insect species investigated were Callosobruchus maculatus F., Sitophilus zeamais Motsch, Dermestes maculatus Deg. Stock cultures of insecticide susceptible strains of these species were obtained from the Tropical Development and Research Institute (TDRI) at Slough where they had been maintained in culture for several years.

Table 2.1 Sources of oils tested for insecticidal activity.

Oil[+]	Extraction method	Country of origin	Immediate source of extracted oil.
Groundnut oil	Hot press (industrial)	Nigeria	Lagos company
Palm oil	Hot water process of pulp (industrial)	Unknown	Local U.K. supermarket
Traditional palm oil	Pulp extracted with hot water (traditional)	Nigeria	Benin (family press)
Coconut oil	Hotpress (industrial)	West Indies	Local U.K. supermarket
Traditional coconut oil	Roasting and hot pressing	Nigeria	Benin (family press)
Sunflower oil	Unknown (industrial)	Unknown	Local U.K. supermarket
Corn oil	Hotpress (industrial)	Unknown	Local U.K. supermarket
Sesame oil	Unknown (industrial)	Unknown	Local U.K. supermarket
Sharkliver oil	Hot water	Senegal	TDRI (Slough)
Limepeel oil	Steam distilled (industrial)	West Indies	Zimmerman Hobbs. U.K.
Lemonpeel oil	Machine press (industrial)	Sicily	" "
Sweet Orangepeel oil	" "	Brazil	" "
Tangerinepeel oil	" "	Sicily	" "
Grapefruitpeel oil	" "		" "
Mandarinpeel oil	" "		" "

[+] 1-9 are fixed oils; 10-15 are essential oils.

2.1.3. Insect Culture

All species were kept in a constant temperature (CT) room at 28° C and approximately 70% R.H., under constant red light. Since insects are not sensitive to this part of the spectrum, insects were maintained under effectively dark conditions thus simulating typical storage conditions. Culture jars were held in plastic dishes placed on trays filled with paraffin oil to prevent mite infestations. All bioassays were carried out in the CT room. The insects were cultured using the following procedures:

1) C.maculatus. Stock cultures were reared on blackeyed beans (Cowpea). About 60 young adult insects (mixed sexes) were placed in sterilized 880 ml glass jam jars containing approximately 350 g of beans. The jars were covered with a Whatman No.1 filter paper which was sealed with paraffin wax, and then covered with muslin secured by a rubber band. To ensure a continuous supply of adults, insects were recultured every six days.

2) S.zeamais. New cultures were prepared by removing about 150 adults (mixed sexes) from an older stock culture and placing them on 350 g of wheat in a glass jar as above. After a 2-3 week oviposition period, the adult beetles were removed by sieving and the grains replaced. The insects were recultured every 21 days and older cultures were discarded.

3) D.maculatus. This species was maintained in a Kilner jar (1 lb). A wad of wet cotton wool (free water encourages oviposition in D.maculatus - Osuji, 1975) was placed at the bottom of the

Kilner jar together with a fishmeal (400 g) and dried yeast powder (25 g) mixture. In order to increase the nutritive value of the culture medium, 2 or 3 slices of raw bacon were placed on top of the fish meal. About 50 young adult beetles (mixed sexes) were then added to the Kilner jars and each jar covered with filter paper and muslin held down with wax and a rubber band respectively. After 3 weeks the adults were removed from the culture medium and the jars resealed. New cultures were set up once every 4 weeks.

2.1.4 Experimental Insects

Fresh cultures were sieved (mesh size 10) to remove all adults since they were of uncertain age. The day of sieving was taken as day 0 and after the newly emerged insects had reached the appropriate age the cultures were sieved again to remove adults of a known age range. During sieving, C.maculatus and S.zeamais adults passed through the sieves and were caught in the bottom pan while the grains used in the culture were retained. With D.maculatus the sieve allowed the fishmeal together with first and second instar larvae to pass into the collection pan, while the adults and larger instars were retained. The adults were then transferred with fine forceps into glass beakers.

2.1.5 Sexing of Insects

The sex of each beetle was determined using the characteristics described by Halstead (1963) for C.maculatus and S.zeamais, and by Hinton (1945) for D.maculatus. With S.zeamais and D.maculatus, a binocular microscope (x 12.5 magnification) was required to

distinguish between males and females.

2.1.6 Stored Produce

Pesticide-free Cowpea (Black-eyed beans - Vigna unguiculata Walp) and wheat grains (Triticum sp.) both of unknown variety were obtained from Oasis Wholefoods, Windsor. Both were stored in polythene bags at 5°C to prevent insect infestation. Before use, batches of grains were transferred to large Kilner jars (1 lb), covered with muslin and kept in the experimental CT room for 2-3 weeks to allow the grains to equilibriate to the ambient RH.

Dried rainbow trout (Salmo gairdneri) was obtained from a London fishmonger and dried at TDRI (London). This fish was chosen because it is an easily available species in the U.K. and it is a reasonable equivalent to most of the commercial dried fish in Nigeria which are mainly freshwater (Osuji, 1973). Before drying, each fish was gutted, the bones and head removed, and the fish filleted into 3 or 4 fairly equal strips. These fish strips were then arranged in a single layer, skin downward on trays in a ventilated Afos mini kiln at 25°C for 7 days. Slow drying of the fish ensured further standardisation of the muscle strips as such procedures are known to have the least effect on the texture and chemistry of the fish (Dr. C. Wood, TDRI, personal communication). After drying, the moisture content of the fish strips was approximately 10%. Dried fish was left in plastic boxes in the experimental CT room for 3-4 weeks to allow them to equilibriate. The moisture content of equilibriated dried fish was about 15%.

2.2 Oil Application and Bioassay Procedures

2.2.1 Application to Grains.

2.2.1.1 Choice of equipment.

The relative efficacy of three methods of mixing oil and grains (Griffin flask shaker, Laboratory tumbler and manual mixing) was evaluated visually after mixing the same quantities of dyed oil and cowpea/wheat grains for 10, 20 or 30 min. Red dye O, an oil soluble colourant (French, 1926), was used for both groundnut and Limepeel oils giving a deep red colour in solution.

With manual shaking, a good spread of oil was only obtained after highly variable mixing times depending on the efficiency of the operator. Both mechanical devices gave satisfactory spread of dyed oil on cowpea/wheat but the flask shaker gave an equivalent spread of oil to the tumbler in half the time (10 min.). In addition, the tumbler used could only take one sample of grains at a time compared with 6 samples in the flask shaker. The Griffin flask shaker was therefore used for mixing oil and grains in the subsequent experiments.

2.2.1.2 Standardisation of mixing grain and oils.

a) Introduction of oil: the same quantity of dyed groundnut oil was pipetted onto 20 g batches of cowpea or wheat either before or a few seconds after shaking commenced.

b) Shaker speed: with the same amount of dyed oil (0.28 ml/20 g cowpea; = 14ml/Kg) the flask shaker was run at various speed settings (5, 6, 7, 8 or 9) for 10 min. At the end of each

operation, the beans were checked for damage and oil spread.

c) Shaking time: after determination of the best shaker speed, groundnut and limepeel oils were stained with red dye O and each oil was applied to 20 g batches of a known number of grains of cowpea or wheat. The grains were then shaken for 5, 10, 15, 25 or 35 min. with 2-3 dosages of oil (3.5, 7 or 14 ml/Kg). Each treatment was replicated twice. At the end of each mixing operation, the grains were quickly transferred to a clean 25 ml beaker and the dyed oil extracted with absolute ethyl alcohol (10 ml) for 2h. The alcohol extract was then measured spectrophotometrically using a Beckman D.B. grating spectrophotometer at 520 nm; the amount of dye present being determined from a standard calibration curve.

d) Spread of dyed oil: since the bioassays were to be conducted with a fixed number of grains taken from a treated batch or from several such batches, the uniformity of dyed oil within and between batches of grains was assessed. Dyed groundnut oil (3.5 and 14ml/Kg) was introduced during shaking onto batches of cowpea or wheat (20 g) which were left shaking for a further 15 min. Three batches of 20 grains of cowpea or 5g portions of wheat were then taken from each treatment and the amount of dye deposited on the grains determined as described above. The experiment was repeated with oil at the higher rate.

2.2.1.3 Results.

a) Introduction of oil. Visual inspection showed that pipetting oil onto grains before shaking commenced resulted in a few grains being covered with a much greater amount of dye than the

remainder. Where dyed oil was introduced after shaking had commenced there was a visibly uniform spread of dye.

b) Shaker speed. The spread of dyed oil on cowpea grains increased with shaker speed. However, at settings 8 and 9 the grains were agitated too violently leading to splashing of oil and grain damage, while settings 5 and 6 were too slow, requiring long periods (>20 min.) to produce an acceptible spread of dyed oil among the grains. On the other hand, setting 7 (1100 oscillations/min.) gave a uniform spread of oil on grains (10-40 g) within 20 min. with no grain damage and was therefore selected for use in bioassays.

c) Shaking time. The amount of dye picked up with time at each dosage level per grain (DPG) of cowpea or wheat is shown in Fig. 2.1. The continuous lines represent the equations from a linear regression analysis for DPG against Log. (1/time), replotted on a normal time scale. In each case, the relationship between the dye pick up per grain (DPG) and the mixing time was asymptotic with the curves levelling out after approximately 15 min. This was therefore chosen as the time to achieve optimum spread of oil.

Multiple regression of dosage (level of dyed oil applied to grains) and DPG against Log. (1/time), replotted in Fig. 2.2 on a normal time scale, shows that the DPG was dosage dependent. While the rate of increase of DPG appeared similar at the three dosage levels tested. The validity of the multiple regression analysis depends on the assumption that parallel lines were an appropriate representation for the time course of dye uptake at the 3 dosage

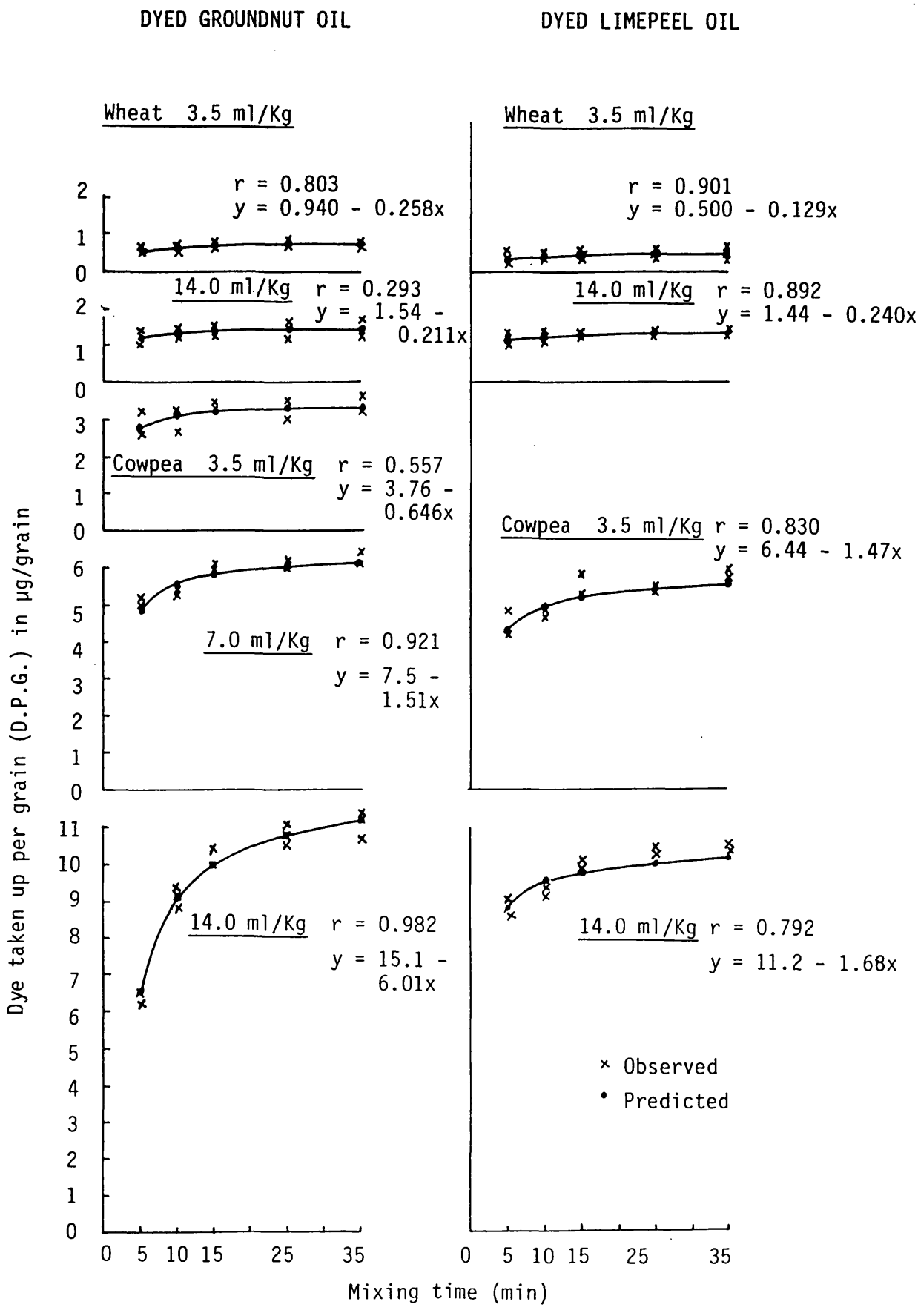


Fig. 2.1 Uptake of dyed oils on cowpea or wheat with shaking time (regression lines plotted on natural time).

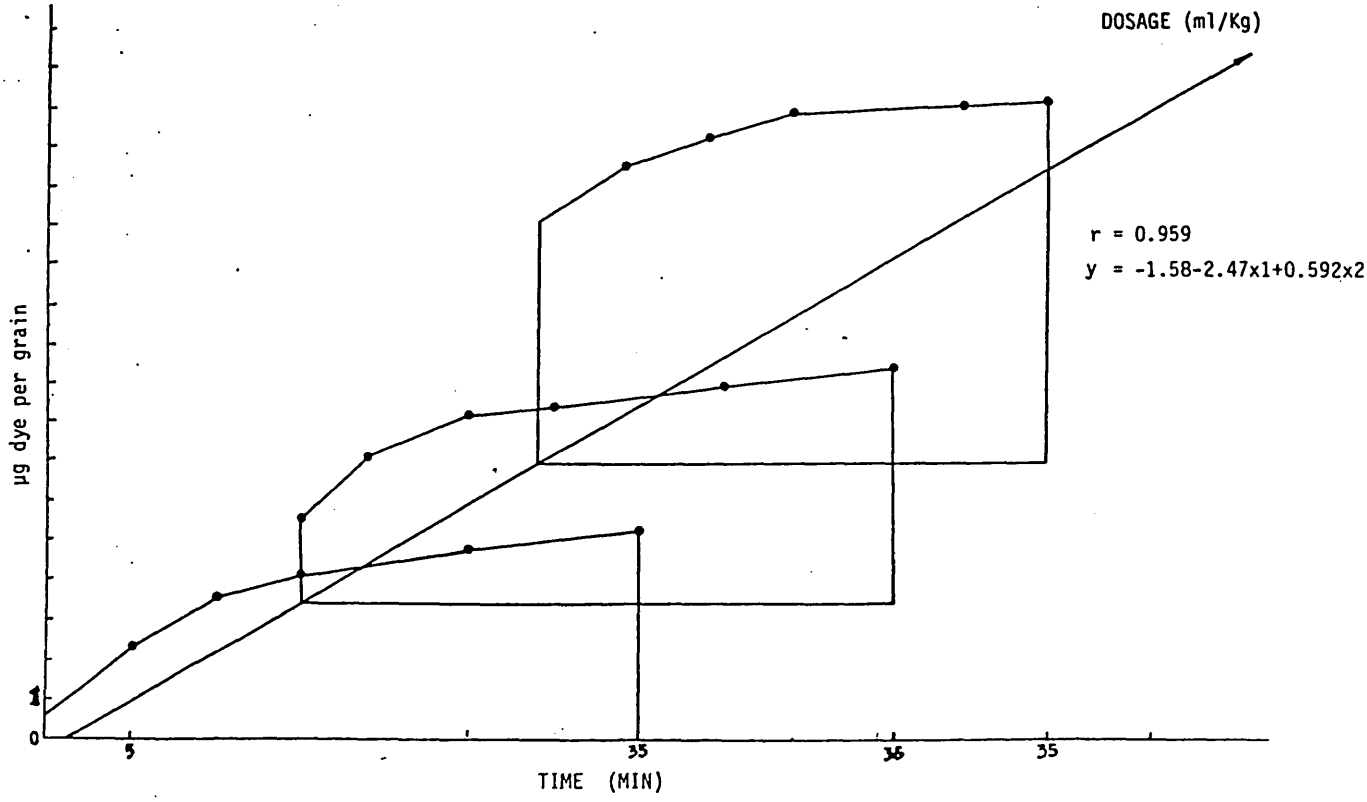


Fig. 2.2 Multiple regression analysis (replotted on a natural time scale) of uptake rate of dyed oil applied at varying rates to cowpea grains in a shaker.

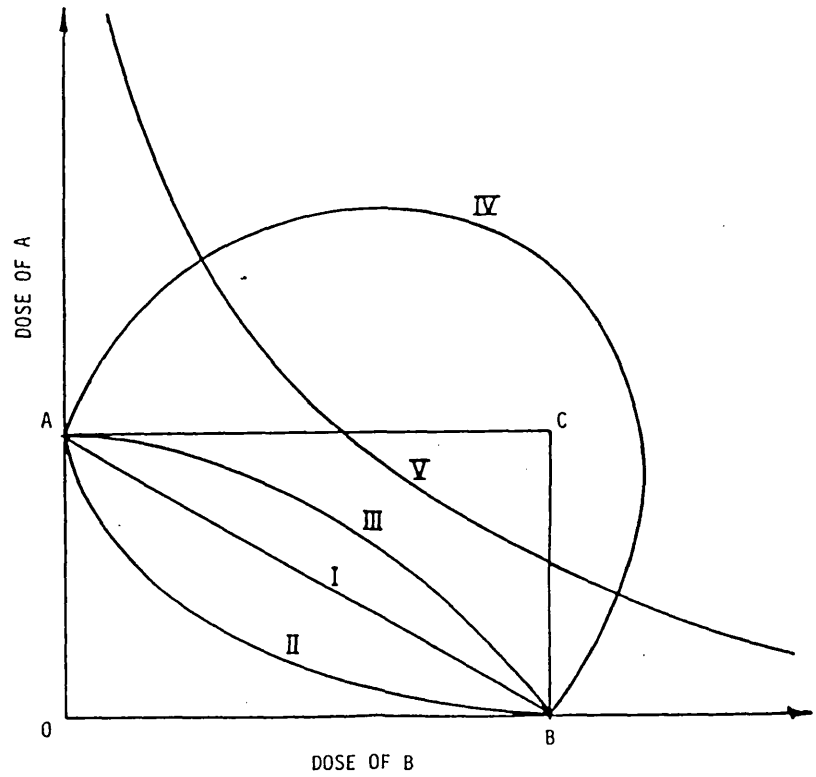


Fig. 2.3 Isoboles where both drugs are separately active (I-IV) or where both are separately inactive (V). After Hewlett and Plackett (1979).

levels tested. However, it appears from Fig. 2.2 that the slopes may change with dose and a statistical modelling analysis was therefore conducted to check the validity of the parallel line fit. Table 2.2 gives the results obtained in summary form. Clearly no significant improvement in the fit of the model resulted from adding the [Log. (1/time)]-dosage interaction term, which represents non-parallel lines for the 3 doses. It was thus reasonable to conclude that differences between the slopes were not statistically significant ($P>0.05$), and that the use of the multiple regression formula to predict uptake rates on the basis of dose level and mixing time was valid.

d) Spread of dyed oil. The variations in the amount of dyed oil picked up per replicate for the same treatment batches with identical mixing regimes are shown in Table 2.3. A one way analysis of variance gave an F ratio of 39.37 ($P<0.001$), which shows that the variation between treatments was almost 40x greater than the variation between replicates ("within treatments"). It was concluded therefore that the experimental procedures used were extremely reproducible. The amounts of dye picked up in replicates of cowpea or wheat grains from different batches of grains treated at the same dosage were also not significantly different ($P>0.05$; see Table 2.3).

2.2.1.4 Summary of standard procedure for application of oil. A Griffin flask shaker was used to mix 10-40 g batches of grains at setting No. 7 ($\frac{1100}{}$ oscillations/min.) for 15 min. Measured amounts of test oils were introduced a few seconds after commencing shaking.

Table 2.2 Summary of statistical model including ANOVA for uptake rate of dyed oil applied at varying dosages onto grains in a shaking system.

Model	Effects	D.F.	SS.	D.F.	SS.	Change	F-ratio
Total	Fit grand mean	29	234.58				
Add doses	Fit separate means	27	32.11	2	202.47	101.24	138.06*
Add Log (1/time)	Fit parallel lines	26	19.06	1	13.04	13.04	17.79*
Add Log (1/time). Doses	Fit non-parallel lines	24	17.63	2	1.43	0.72	0.98N.S.

* Significant ($P<0.001$).

N.S. Not significant ($P>0.05$).

Table 2.3 Effect of the standard mixing regime on the spread of dyed oil on cowpea and wheat grains: variations between treatments and within treatments.

Treatments (ml|Kg.)	* $\bar{x}$ amounts of dye deposited per grain portion ± S.E.
Cowpea (14)	1. 55.67 ± 2.65
	2. 51.33 ± 1.20
Cowpea (3.5)	33.33 ± 2.06
Wheat (14)	1. 60.17 ± 1.96
	2. 57.67 ± 1.48
Wheat (3.5)	37.00 ± 1.18

n = 3

L.S.D. (at $P<0.05$) = 5.2

* = ($P<0.001$).

A statistical modelling approach established that the multiple regression formula given below can be used to predict uptake rates of oil on the basis of dose level and mixing time:

$$y = -1.58 - 2.47 \times 1 + 0.592 \times 2$$

where y = uptake rate

x1 = Log 10 (1/time)

x2 = dosage level.

2.2.2 Application to dried fish strips.

2.2.2.1 Evaluation of different methods.

The dried fish strips used in this study had a mean surface area of 23.6 cm^2 (range 11.3 - 36.0 cm ; n = 50) and were usually less than 1.0 cm thick (Plate 1). Due to this leaf-like structure, oil could not be applied uniformly using a shaker or tumbler. The methods of application investigated were as follows:

a. Spraying.

b. Dipping.

c. Manual brushing.

a. Spraying using a Potter tower was found to be unsuitable because the nozzle and connecting tubes retained varying different amounts of dyed oils depending upon their viscocity. This made calibration and reproducibility difficult. Also, having to spray each fish strip twice in order to cover the two main surfaces was time consuming.

b. Dipping was also unsatisfactory as some of the test compounds (notably essential oils) were only available in insufficient amounts for this method. In addition, dosage determination with fish strips dipped in dyed groundnut oil was difficult and the

variability between treated batches was unacceptably large.
c. Brushing of oil onto all visible surfaces of dried fish strips was found to be the most efficient and convenient method. The procedure was standardised using dyed (Red dye O) groundnut oil which was then extracted and quantified spectrophotometrically as described earlier.

It was found that with only one person brushing, uniformity of oil spread could be achieved within and between treatments. When brushing care must be taken to cover all surfaces including edges and crevices in the fish muscle. To minimise the loss of measured quantities of dyed oil, it was necessary to pipette the oil directly into the bioassay container prior to brushing onto the fish strip(s). The weight of fish strips was standardised for each experiment and the number of separate strips per replicate reduced to a minimum. Spread of oil varied with the size and texture of the brush used. A camel's hair brush (size 3) was found to be the most suitable and convenient. This brush was large enough to allow rapid coverage of the fish strips but small enough to reduce loss of oil adhering to brush hairs to a minimum. One brush per replicate was used to prevent carry-over of oil.

2.2.2.2 Summary of standard procedure for application of oil.
The required amount of oil was measured into the bioassay container, from where it was picked up and applied to the fish strip using a size 3 camel hair brush, covering all exposed surfaces.

2.2.3 Bioassay procedures.

2.2.3.1 Bioassay container.

Plastic and glass laboratory containers were tested for their reactivity with plant oils by adding a few drops of all oils to be tested and observing them over 24 h. It was found that all essential oils reacted with plastic containers, corroding them almost immediately after contact. Fixed oils had no such effect. Therefore, only glass containers were employed in experiments involving essential oils.

Prior to bioassay, the sides of the test containers were coated with Fluon to prevent insects crawling up or escaping.

2.2.3.2 Procedures prior to bioassay.

Equilibriated cowpea or wheat grains were spread on clean trays in order to pick out broken grains and shafts before weighing on a top-loading Mettler balance. Dried fish strips were similarly weighed out into the required portions using cut pieces to make up the required weight when necessary. Oils were then applied to grains or fish strips using standard application methods. Standard amounts of treated or untreated grains/fish were enclosed in the bioassay containers and kept in the experimental CT room for a fixed period prior to bioassay. In all experiments the treatments were fully randomised.

2.2.3.3 Bioassays.

a. Adults. Fully active insects of known age from the same generation were removed from culture jars (Section 2.1.4) and if necessary sexed (Section 2.1.5). The appropriate number of insects was then placed into glass vials (35ml; 7.5 x 2.5 cm)

with a ventilated cork stopper covered with muslin and kept in the CT room until bioassay. The insects were inspected before release into the test container and any dead or moribund insects replaced from a similar age group. Adult insects were involved in two main types of bioassays:

1. Assessment of activity of plant oils on progeny development. In such experiments, pairs of young adult males and females (age specified for each experiment) were confined with treated or untreated grains/fish for various times. On removal of the adults, their progeny were assessed as described in later sections.

2. Mortality-dosage experiments. Populations of insects (usually mixed sexes) were exposed to various treatments for different times. Insects were taken as dead if they did not move away when touched gently or tilted.

b. Eggs and larvae. With C.maculatus, sixteen, 0-1 day old adults (8♀ + 8♂) were confined with 40 uninfested cowpea grains for 24 h (initial trials showed that this resulted in an even spread of eggs on >90 % of grains). The infested grains were left in perforated plastic containers in the CT room until bioassay as eggs or larvae. Larvae were counted ten days after oviposition (hatching occurred at 4-7 days); each hatched egg indicating a live larva within the grain (untreated grains with 100 hatched eggs had 98 live larvae). Unhatched C.maculatus eggs were easily recognised as grey dome-shaped structures with oval, flat bases (P. Dobie, C.P.Haines, R.J. Hodges and P.F. Prevett, TDRI Training Manual); hatched eggs were white in colour with no dark spot.

With S.zeamais, seventy, 4-14 day-old adults (mixed sexes) were confined with 150 g of uninfested wheat for 10 days. On removal of adults, the grains were left with 0-10 day-old immature stages (eggs and early instar larvae). In preliminary trials, where grains were similarly infested, X-ray radiographs (see below) of 50 grains revealed immature stages in more than 70 % of grains. Measured amounts of infested grains were used in subsequent bioassays.

With D.maculatus, twenty, 0-15 day-old adults (mixed sexes) were confined in plastic containers with pieces of double thickness Kimwipe as a substrate for oviposition. The insects were provided with small pieces of dried fish as food and 10 drops of water per day. Eggs were usually laid between the double layers of the paper; eggs were transferred to bioassay containers with a camel's hair brush.

c. Oviposition experiments. With C.maculatus, oviposition was assessed by counting the eggs laid on the cowpea grains (using a binocular microscope at x 12.5) after removal of adult beetles. The grains were then transferred to ventilated glass vials.

With S.zeamais, oviposition and development of immature stages within grains was assessed using an X-ray radiographic technique. The grains in each replicate were attached in rows on single-sided Sellotape in a plastic frame; the grain crease pointing downwards with the germ towards the bottom row (Plate 2). On pre-determined dates, X-ray radiographs were taken with a Hewlett Packard 43804N X-ray system (Faxitron series) with a tube

voltage of 12 KVp, a maximum tube current of about 3 mA and an exposure time of 4.5 min. Kodak Industrex Mx X-ray film was used; being developed and fixed immediately after exposure. Two X-ray radiographs of each batch of grains were taken as follows: at 16-17 days and at 29-30 days of the bioassay. The X-ray films were examined under a binocular microscope (x 8) and by superimposing the pairs of X-ray films for each batch of grains, the number of eggs laid, the number of live larvae and the number of dead larvae were counted.

With D.maculatus, Preliminary trials showed that finding and counting eggs was not practical as the eggs hatched within 3-4 days and they were often concealed within the fish muscle and thus missed or destroyed in the pre-assessment search. Instead, larval emergence was assessed when larvae were 8-18 days old. Larvae were carefully collected from fish strips by examining all surfaces, including muscle crevices and underneath the skin. A binocular microscope (x 12.5) was used to pick out the smaller larvae. The fish strips, together with all the larvae found on them, were then transferred to a honey jar (355 ml) covered with a Whatman No.1 filter paper (6 cm dia.) and secured by a screw top with a 5.4 cm dia. ventilation hole (ventilated honey jar).

d. Assessment of adult emergence. With C.maculatus, grains were observed daily from the time emergence "windows" were first observed. Emergent adults were removed, counted and discarded. A similar procedure was used for S.zeamais except there were no "windows" to indicate emergence. With D.maculatus, once emergence started each honey jar was examined every few days. Sieving was used to remove any adult insects; immature stages and fish pieces

being returned to the honey jar after examination.

Since emerged adults may have had a chance to oviposit before removal, assessment of emerged adults was stopped for all 3 species just before the generation time was exceeded (counted from the date of first emergence).

e. Topical application of oils to D.maculatus. Test oils were applied to the prothoracic region of adults (0-14 day-old) or to the mid-dorsal abdominal region of larvae (21-28 day-old) using an Arnold microapplicator fitted with a glass Aglar syringe (1 ml). Twenty or thirty insects were dosed per treatment. The treated insects were not provided with food. Control insects were handled similarly but not dosed. Mortality was assessed at 24 h intervals.

f. Dipping of C.maculatus eggs. Egg-infested grains were dipped in a solution of the test oil in acetone and removed in one movement. The treated grains were dried on a filter paper for 5 min. and then placed in a Petri dish. Five grains with a total of about 20 eggs were used per treatment replicate. Control eggs were dipped in acetone. Only 0-3 day-old eggs were tested in this way as preliminary trials showed that acetone was harmful to older eggs.

g. Fumigation experiments. The fumigant action of essential oils was assessed in air-tight Kilner jars (500 ml) sealed with a screw ring holding a glass lid onto a rubber washer (covered with aluminium foil to prevent any reaction with test compounds). This system was adopted because of its simplicity (Busvine, 1971) and

ease of operation.

The test oils were dispensed by glass microcaps (Drumond) or Jencons Finnpipette (for volumes greater than 5 µl) onto a Whatman No.1 filter paper (4 cm dia.). Preliminary experiments showed that all test compounds could readily evaporate from the filter paper when this was suspended on a string within the chamber. Solid compounds of known weight were applied to a small wad of cotton wool which was then suspended inside the chamber. The oils were applied to the chamber after addition of the insects (usually 15 per chamber); the stopper being quickly replaced to minimise loss of volatiles. Insects were confined to the bottom third of the chamber by coating the sides of the chambers with Fluon and were not provided with food during fumigation.

2.2.4 Statistical analysis

a) The main computer statistical packages employed were:

1. Minitab (Ryan et al., 1981; Pennsylvania State University).
2. Genstat (Genstat V copyright, 1980; Lawes Agricultural Trust, Rothamsted Experimental Station).
3. Probit analysis for single lines and test for parallelism and relative potency based on Finney (1971). Relative potency based on LC_{50} ratios were shown as the toxicity factor (TF) in the text.

When ANOVA was used, unless otherwise stated, the first test consisted of comparing all treatment means including the control. Further analysis (main interaction effects, comparing individual means with control etc.) was carried out only if there was a significant difference at the 5 % level ($P<0.05$). Standard errors (S.E.) were based on the pooled standard deviation.

b) The joint fumigant action of citrus oil components. A general equation was used to calculate the expected dose (Z) of a mixture of insecticidal components which will produce some specified response in a target population:

$$\frac{C(1)}{Z(1)} + \frac{C(2)}{Z(2)} + \frac{C(3)}{Z(3)} - - - + \frac{C(n)}{Z(n)} = \frac{1}{Z}$$

After Hewlett and Plackett (1979).

where C(1) is the proportion of component 1 in the mixture and Z(1) is the dose of component 1 required to produce the same effect in the target population.

The above equation depends on the assumption of additive action (Hewlett and Plackett, 1979). The LC_{50}'s for all insecticidal components of citrus oil tested in this work were determined by probit analysis and their proportions in the citrus oil determined by gas chromatography or obtained from the literature (Shaw, 1979). The LC_{50} of a typical citrus oil (limepeel) was similarly determined. An estimate was then made of the toxicity of the mixture of components not individually examined, by substituting known LC_{50} values and proportions into the above

equation.

c) The joint action of binary mixtures of citrus oil components. Several models have been developed to classify and quantify joint action between two or more compounds, one or both of which may be individually active (Hewlett and Plackett, 1979). Two models were adopted in the analysis of data described below:

1. The joint action between toxic components of citrus oils was presented in the form of isobolograms; an isobologram for two toxicants consisting of a curve which at any point represents the dose-pair for a chosen level of response (in this work the LC_{50}). Isoboles have been used in pharmacology for about one hundred years (Loewe and Muishnek, 1926; Loewe, 1953; 1959). However, the terminology has not been fully standardized and in the present work that of Ariens et al. (1976) is used.

   Fig. 2.3 shows isoboles for two drugs A and B; points on each isobole (i-v) representing the amounts of A and B in a formulation which produce a given biological response (usually the LC_{50} or LD_{50}). Points A and B represent the amounts of drugs A and B which individually produce the biological response. Mixtures producing isobole I, a straight line, have additive action. Isobole II, lying below the line A-B, represents potentiation or synergism. Isobole III, lying above the line AB but within the area A-B-C, represents sub-additive action. Isobole IV, which

proceeds from A to B but lies above and to the right of the area A-B-C, represents antagonism. Isobole V, where neither drug is separately active, represents coalitive action.

2. Plackett and Hewlett (1963) described a method for testing the hypothesis that bioassay data for two insecticides is consistent with a model of completely similar joint action. A special case of the model, that of two compounds acting similarly but with non-parallel Log. dose/probit regression lines, gives rise to the equation:

$$h(x(1) - x)/b(1) + h(x(2) - x)/b(2) = 1$$

where b(1) and b(2) are the slopes of the Log. dose/probit regression lines of the components bioassayed singly; x(1) and x(2) are the normal equivalent deviates (n.e.d) of the responses of the test organism to the doses of the mixture components when applied separately, and x is the n.e.d. of the response to the components applied as a mixture.

The method has been implemented as a BASIC programme (G.N.J. le Patourel, unpublished) and requires bioassay data for the components applied singly and as a mixture to the test population. The fit of the data to maximum likelihood estimates of the Log. dose/n.e.d. response lines for the individual components is tested by assuming a Chi-squared error distribution. A

non-significant Chi-squared value implies that the data is consistent with the above model of joint action.

ACTIVITY OF FIXED AND CITRUS ESSENTIAL OILS AGAINST PROGENY DEVELOPMENT

3.1 Introduction

A number of workers have demonstrated that plant oils can effectively control grain beetles in laboratory bioassays (Section 1.2) and it has been suggested that these oils can be used to control such pests in storage systems. However, the relative insecticidal activity of the plant oils tested on grains varies greatly in the literature and no attempt has been made to fully describe and compare the activity of fixed and essential oils; groups which have been considered to act similarly (Golob and Webley, 1980; Mensina and Renwick, 1983; Su et al., 1972b). The possible effects of oils in protecting dried fish has also not been evaluated. In view of this, the present work began by screening 8 fixed oils (vegetable oils) and 6 essential oils (citrus peel oils) for biological activity against C.maculatus on cowpea, S.zeamais on wheat and D.maculatus on dried fish.

In order to test the reproducibility of the screening experiments, several oils were retested against each insect species. The repeat experiments were also aimed at providing statistical validity for the observations made during the preliminary experiments.

The oils tested in secondary experiments were chosen on the basis of their activity and availability particularly in tropical countries where indigenous compounds could play an important role in the control of stored product pests. Sharkliver oil was also

tested to see if animal fixed oils have a similar insecticidal effect to plant fixed oils.

3.2 Materials and methods

3.2.1 Primary screening of fixed and essential oils

3.2.1.1 C.maculatus.

Oils were applied at 3.5 and 14 ml/Kg seed (Section 2.2.1.4). Immediately after application, 20 treated or untreated cowpea grains were placed in a glass Petri dish (7 cm dia.). Two sets of controls were used, shaken (as in test grains) and unshaken. About 10 h after application, four 1-4 day-old adults (2♀, 2♂) were placed in each Petri dish and left for 5 days. All treatments were replicated 3 times. The activity of the oils were assessed by estimating oviposition and adult emergence (Section 2.2.3.3.).

3.2.1.2 S.zeamais.

Test oils were applied to 20 g batches of wheat grains at 3.5 and 14 ml/Kg (Section 2.2.1.4). Immediately after application, 5 g ($\bar{x}$ of 128 grains) of treated or untreated grains were spread out in a glass Petri dish (7 cm dia.). About 18 h after application, six 3-9 day-old adults (3♀, 3♂) were placed in each Petri dish and left for 10 days. All treatments were replicated 3 times. After the removal of adults, the grains along with any frass were transferred to ventilated vials to prevent emergent adults escaping. The experiment was assessed by measuring adult progeny emergence (Section 2.2.3.3) which continued until the 59th day of bioassay, approximately 3 weeks after first adult

emergence.

3.2.1.3 D.maculatus.

Test oils were applied to 15 g portions of dried fish strips (Section 2.2.2.2). The higher of the two rates of oils tested (28 ml/Kg fish) was taken to approximate to the amount of oil that gave complete coverage of a dyed oil film over dried fish. This would be approximately equivalent to the oily layer rubbed on to fish by traditional fishmongers in Nigeria (K. N. Don-Pedro, unpublished observations) which was determined (Section 2.2.2.1) to be 30 ml/Kg. The lower rate chosen was 14 ml/Kg, where oil covered the fish surface only sparingly. About 12 h after application, four 1-16 day-old adults (2♀, 2♂) were placed in each Petri dish and left for 10 days. Each treatment was replicated 3 times. The experiment was evaluated by assessing larval emergence and adult progeny (Section 2.2.3.3).

On the basis of the results from the above experiment, groundnut, limepeel and orangepeel oils were also applied at 56 and 112 ml/Kg to give a more generous oil film. Each treatment was replicated twice.

3.2.2 Secondary trials with selected fixed and essential oils

3.2.2.1 C.maculatus.

The oils tested were groundnut, traditional coconut, sharkliver oil (of similar viscosity to plant fixed oils and with no essences), limepeel, tangerinepeel and grapefruitpeel at 3.5 and 14 ml/Kg (Section 2.2.1.4). About 12 h after application, four 1-2 day-old adults (2♀, 2♂) were placed in each Petri dish with

20 treated or untreated grains for 5 days. All treatments were replicated six times. The bioactivity of the oils was evaluated as described previously (Section 3.2.1.1).

3.2.2.2 S.zeamais.

The oils tested were groundnut, traditional coconut, corn, limepeel, tangerinepeel and grapefruitpeel at 3.5 and 14 ml/Kg (Section 2.2.1.4). About 12 h after application, six 2-10 day-old adults (3♀, 3♂) were placed in a Petri dish with 5 g of treated or untreated wheat grains for 10 days. All treatments were replicated six times. The experiment was assessed by recording adult progeny emergence (Section 3.2.1).

3.2.2.3 D.maculatus.

The oils tested were groundnut, traditional coconut, sharkliver, orangepeel and grapefruitpeel. The fixed and essential oils were applied at 28 and 112, and 28 and 56 ml per Kg respectively. About 14 h after application, four 1-12 day-old adults (2♀, 2♂) were placed in a ventilated honey jar with 15 g of treated or untreated fish strips for 10 days (honey jars were used instead of Petri dishes to provide more space for developing progeny). All treatments were replicated six times. The experiment was evaluated by recording emergent larvae after 18 days and adult progeny emergence (Section 2.2.3.3). In addition, all emergent larvae, together with the fish strips, were weighed using an Oertling balance (day 18). All emergent adults and remaining fish pieces were weighed after 67 days when all F1 adults had emerged.

3.2.3 Rate of loss of biological activity

3.2.3.1 Relative rate of evaporation of fixed and essential oils. In an initial experiment, weighed amounts of limepeel or groundnut oil (1 ml) were added to an aluminium foil container (5 x5 x 0.5 cm). Each container was then placed in an open Petri dish in the CT room. The containers were weighed at hourly intervals for the first 6 h and then once every 24 h for 15 days. In a repeat experiment the oils were enclosed in a Petri dish or a ventilated honey jar.

The rate of evaporation of limepeel and groundnut oil from treated grains in a Petri dish (7 cm dia.) or a ventilated honey jar (355 ml; Sections 3.2.1; 3.2.2) was also evaluated using a bioassay method. Twenty, 1-3 day-old adults of C.maculatus were confined with 20 g of treated or untreated cowpea grains per container. Each treatment was replicated 3 times. Mortality was assessed after 24 h and the number of eggs laid after 5 days determined.

In order to compare the evaporation rate of essential and fixed oils, each oil (0.1 ml) was pipetted onto a pre-weighed Whatman No.1 filter paper (11 cm dia.) which was then suspended in the open laboratory (approx. 18 °C). The filter papers were reweighed at 0, 6 and 24 h.

3.2.3.2 Effect of the time lag between oil application and bioassay.

With C.maculatus, groundnut or limepeel oil was applied to grains at 7 ml/Kg. Twenty treated or untreated grains were confined in Petri dishes with four 1-3 day-old adults (2♀, 2♂) for 5 days. Each treatment was replicated 5 times. For each oil, bioassays

were initiated 1 and 24 h after application of the oil. Bioactivity of the oils was assessed using 1) adult mortality after 24 h; 2) the total number of eggs laid; and 3) adult emergence.

With D.maculatus, limepeel oil was applied to fish strips (10 g) at 28 ml/Kg. Twenty 0-15 day-old adults (mixed sexes) were confined with treated or untreated fish strips for 10 days. Each treatment was replicated 3 times. Bioassays were initiated 1, 24 and 240 h after application of the oil and activity was assessed by 1) adult mortality after 48 h; and 2) the number of emerged larvae after 18 days.

3.3 Results

3.3.1 Primary screening of fixed and essential oils

3.3.1.1 C.maculatus.

Shaking the grains appeared to have no effect on oviposition and adult emergence when the two controls were compared (Table 3.1). Regardless of the rate of application, all fixed oils tested appeared to have little or no effect on oviposition when compared with controls. In contrast, most of the essential oils, with the exception of orangepeel, effectively reduced the number of eggs laid at the higher dose rate (14 ml/Kg) when compared with the controls.

The fixed oils all showed a fairly uniform degree of activity against adult emergence which was greatly reduced compared with the controls (Table 3.1). The percentage adult emergence from

Table 3.1 Effects of fixed and essential oils on oviposition and adult progeny emergence of C.maculatus.

Treatment oils (ml/Kg)		$\bar{x}$ No. of eggs laid ± S.E.	$\bar{x}$ adult emergence ± S.E.	$\bar{x}$ % adult emergence ± S.E.+
Control [1]		130.0 ± 15.0	98.3 ± 9.2	76.5 ± 2.4
Control [2]		129.0 ± 12.0	83.5 ± 12.5	63.8 ± 4.2
FIXED OILS				
Groundnut	(3.5)	96.3 ± 7.8	50.3 ± 10.0	51.7 ± 6.1
	(14)	109.3 ± 26.0	0.67 ± 0.67	0.1 ± 0.25
Coconut	(3.5)	113.0 ± 4.7	69.7 ± 3.0	62.1 ± 2.6
	(14)	104.0 ± 29.5	2.7 ± 2.7	0.8 ± 1.8
Trad. Coconut	(3.5)	90.3 ± 20.2	58.0 ± 15.6	63.8 ± 7.6
	(14)	111.7 ± 12.2	0.3 ± 0.3	0.1 ± 0.25
Palm	(3.5)	110.0 ± 13.5	76.0 ± 8.9	68.7 ± 2.4
	(14)	103.7 ± 14.1	10.7 ± 2.0	10.6 ± 3.9
Trad. palm	(3.5)	123.3 ± 32.1	81.3 ± 20.0	67.1 ± 3.3
	(14)	85.0 ± 12.7	27.0 ± 4.7	32.9 ± 2.5
Corn	(3.5)	123.3 ± 17.1	69.3 ± 12.9	55.2 ± 3.5
	(14)	141.3 ± 15.4	0.3 ± 0.3	0.1 ± 0.15
Sunflower	(3.5)	100.3 ± 37.8	63.7 ± 25.3	62.1 ± 3.4
	(14)	108.7 ± 4.9	16.3 ± 1.9	15.3 ± 1.9
Sesame	(3.5)	100.7 ± 29.2	42.7 ± 11.8	43.0 ± 1.8
	(14)	119.0 ± 1.2	0.0	0.0

ESSENTIAL OILS contd. over .....

Table 3.1 continued.....

Treatment oils (ml/Kg)		$\bar{x}$ No. of eggs laid ± S.E.	$\bar{x}$ adult emergence ± S.E.	$\bar{x}$ % adult emergence ± S.E. +
ESSENTIAL OILS				
Orangepeel	(3.5)	109.3 ± 23.0	76.7 ± 12.0	71.9 ± 4.7
	(14)	122.3 ± 3.5	90.3 ± 7.8	73.5 ± 4.7
Lemonpeel	(3.5)	133.7 ± 13.4	98.0 ± 8.2	73.5 ± 1.6
	(14)	24.0 ± 10.6	10.3 ± 6.6	32.9 ± 14.6
Limepeel	(3.5)	71.7 ± 13.3	55.3 ± 12.4	76.5 ± 6.7
	(14)	0.0	0.0	0.0
Tangerine-peel	(3.5)	119.3 ± 15.5	87.7 ± 5.6	75.0 ± 4.6
	(14)	45.0 ± 19.6	30.3 ± 15.9	39.6 ± 31.5
Grapefruit-peel	(3.5)	107.7 ± 21.5	85.0 ± 16.1	79.4 ± 1.4
	(14)	65.3 ± 10.5	23.0 ± 4.6	36.2 ± 5.8
Mandarinpeel	(3.5)	107.7 ± 11.3	75.0 ± 12.3	73.5 ± 7.7
	(14)	33.7 ± 32.2	22.0 ± 22.0	9.5 ± 18.0

n = 3

+ Percentage data were angularly transformed.

1. Not shaken

2. Shaken

eggs was only effectively reduced at the high dosage rate. This observation was supported by regression analysis for adult emergence plotted against oviposition (Fig. 3.1). At the lower dosage rate, adult emergence was directly related to oviposition ($r = 0.901$) but this was not the case at the higher rate ($r = 0.316$; $P>0.05$) where the oils exerted near maximum effect.

On the other hand, essential oils reduced adult emergence only where there was a reduced number of eggs laid compared with the controls (Table 3.1). Fig. 3.2 shows that at both dosages there was a strong correlation ($r = 0.948$ and 0.930 for high and low dosages respectively) between the number of emergent adults and oviposition.

3.3.1.2 S.zeamais.

The fixed oils tested were found to be almost equally effective in reducing adult emergence when compared with the controls and totally prevented progeny development at the higher dosages in six out of the eight oils (Table 3.2). To a lesser extent, the essential oils also limited the number of emergent adults when compared with the controls; adult emergence being only moderately reduced at the higher dosage rate.

3.3.1.3 D.maculatus.

None of the fixed oils screened had any apparent effect on the number of emergent larvae or on progeny development (Table 3.3). On the other hand, some essential oils, particularly limepeel oil at 28 ml/Kg appeared to reduce the number of emergent larvae compared with the control (Table 3.3).

Fig. 3.1 Regression analysis of adult emergence of C.maculatus against oviposition for fixed oil treatments.

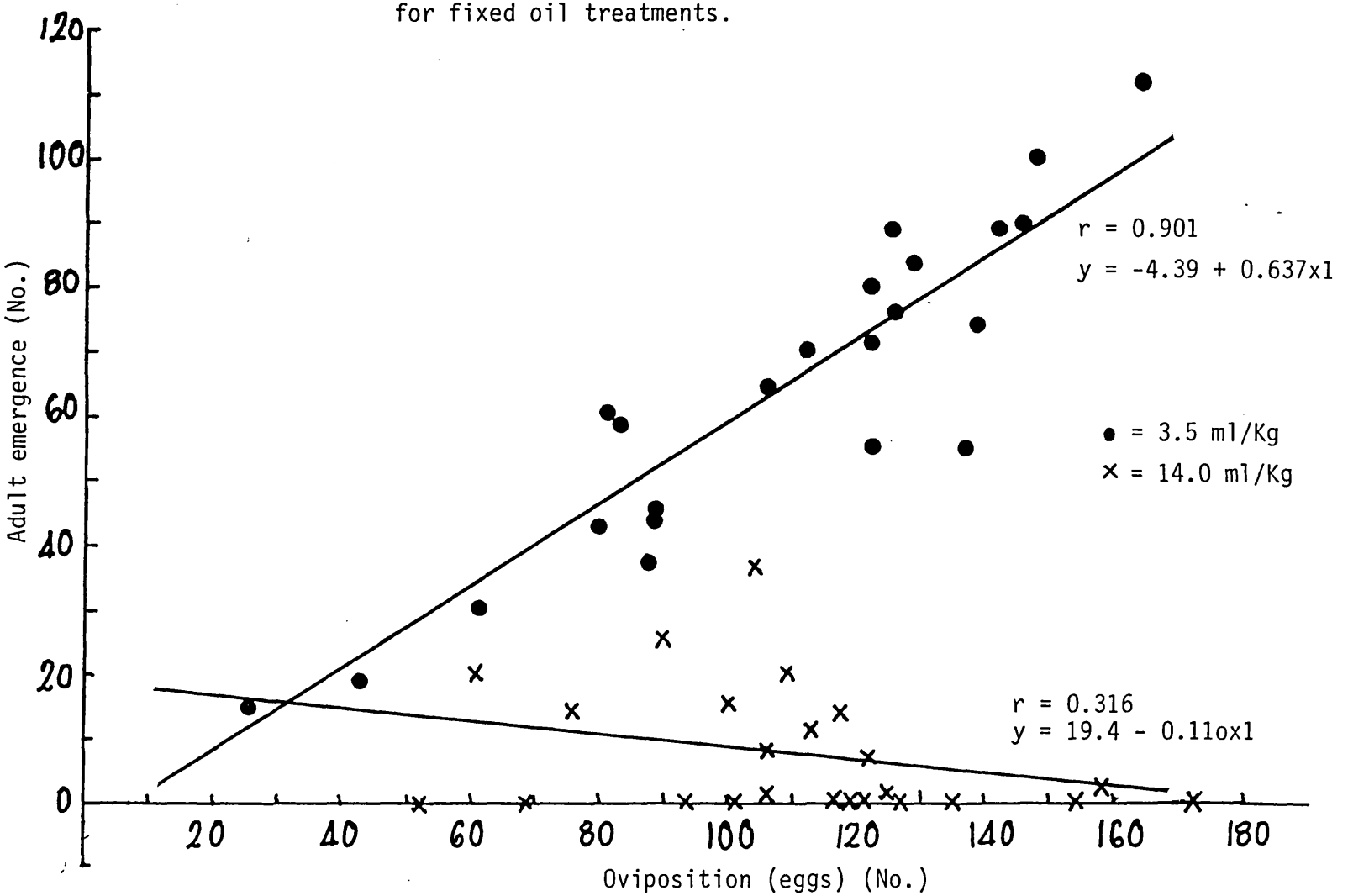


Fig. 3.2 Regression analysis of adult emergence of C.maculatus against oviposition for essential oil treatments.

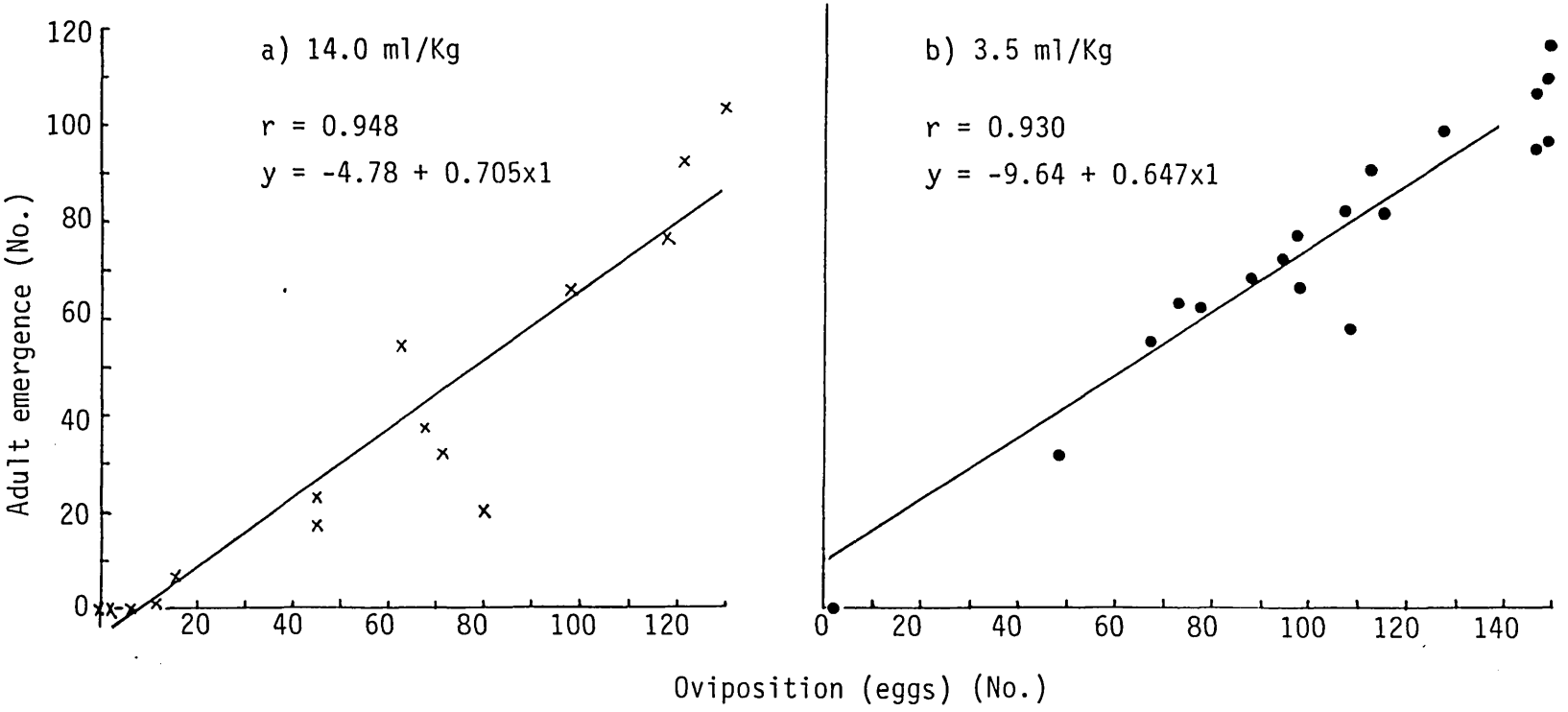


Table 3.2 Effects of fixed and essential oils on adult progeny emergence of S.zeamais.

Treatment oils (ml/Kg)		$\bar{x}$ No. of emergent adults ± S.E.
Control[1]		56.3 ± 1.2
Control[2]		50.3 ± 3.9
FIXED OILS		
Groundnut	(3.5)	23.7 ± 11.0
	(14)	0.0
Coconut	(3.5)	8.3 ± 4.4
	(14)	0.0
Trad. coconut	(3.5)	18.7 ± 9.4
	(14)	0.0
Palm	(3.5)	30.0 ± 13.5
	(14)	2.7 ± 1.2
Trad. palm	(3.5)	34.0 ± 1.5
	(14)	1.3 ± 0.3
Corn	(3.5)	8.7 ± 8.7
	(14)	0.0
Sunflower	(3.5)	18.0 ± 4.2
	(14)	0.0
Sesame	(3.5)	12.0 ± 3.5
	(14)	0.0

Table 3.2 continued over.....

Table 3.2 continued.....

Treatment oils (ml/Kg)		$\bar{x}$ No. of emergent adults ± S.E.
ESSENTIAL OILS		
Orangepeel	(3.5)	42.0 ± 7.0
	(14)	42.3 ± 4.1
Lemonpeel	(3.5)	52.0 ± 7.0
	(14)	16.7 ± 16.7
Limepeel	(3.5)	42.3 ± 5.4
	(14)	10.3 ± 10.3
Tangerinepeel	(3.5)	39.0 ± 6.5
	(14)	18.0 ± 13.3
Grapefruitpeel	(3.5)	26.0 ± 18.0
	(14)	24.0 ± 8.7
Mandarinpeel	(3.5)	30.7 ± 13.9
	(14)	10.0 ± 10.0

n = 3

1. Unshaken

2. Shaken

Table 3.3 Effects of fixed and essential oils on larval and adult progeny emergence of D.maculatus.

Treatment oils (ml/Kg)		$\bar{x}$ No. of emergent larvae ± S.E.	$\bar{x}$ No. of emergent adults ± S.E.	$\bar{x}$ % adult emergence ± S.E.+
Control		86.7 ± 1.5	64.0 ± 12.5	75.0 ± 12.6
FIXED OILS				
Groundnut	(14)	63.7 ± 6.8	49.3 ± 9.3	76.5 ± 7.5
	(28)	80.7 ± 5.8	55.7 ± 11.3	68.7 ± 9.7
Coconut	(14)	85.0 ± 13.5	58.0 ± 14.0	67.1 ± 6.6
	(28)	61.3 ± 14.2	36.7 ± 3.9	65.5 ± 10.8
Traditional coocnut	(14)	78.0 ± 13.3	51.3 ± 13.5	63.8 ± 10.0
	(28)	60.7 ± 12.8	42.0 ± 14.4	70.3 ± 14.8
Palm	(14)	69.0 ± 4.7	52.3 ± 7.8	75.0 ± 6.7
	(28)	68.0 ± 7.1	38.3 ± 3.3	58.7 ± 11.0
Traditional palm	(14)	77.0 ± 7.6	56.3 ± 4.5	73.5 ± 3.1
	(28)	81.7 ± 8.6	60.7 ± 11.6	73.5 ± 7.7
Corn	(14)	85.7 ± 8.4	56.3 ± 4.7	65.5 ± 2.5
	(28)	76.0 ± 14.4	61.3 ± 16.4	80.8 ± 7.7
Sunflower	(14)	80.0 ± 22.5	61.7 ± 22.9	71.9 ± 10.9
	(28)	65.3 ± 20.5	51.0 ± 19.4	75.0 ± 8.3
Sesame	(14)	77.7 ± 9.3	56.3 ± 6.4	71.9 ± 0.8
	(28)	69.3 ± 10.7	56.7 ± 6.5	78.0 ± 7.9

Essential oils continued over.....

Table 3.3. continued.....

Treatment oils (ml/Kg)		$\bar{x}$ No. of emergent larvae ± S.E.	$\bar{x}$ No. of emergent adults ± S.E.	$\bar{x}$ % adult emergence ± S.E. [+]
ESSENTIAL OILS				
Orangepeel	(14)	88.3 ± 14.7	54.3 ± 12.1	60.4 ± 6.0
	(28)	68.7 ± 12.4	55.7 ± 15.6	79.4 ± 10.6
Lemonpeel	(14)	90.3 ± 10.7	52.3 ± 2.0	58.7 ± 6.0
	(28)	76.0 ± 14.9	62.7 ± 13.5	82.1 ± 4.0
Limepeel	(14)	70.0 ± 12.5	42.7 ± 5.4	63.8 ± 10.8
	(28)	22.0 ± 13.7	15.0 ± 10.8	62.1 ± 11.8
Tangerine-peel	(14)	61.0 ± 7.0	52.0 ± 7.2	84.7 ± 3.1
	(28)	79.7 ± 4.3	52.7 ± 12.8	65.5 ± 13.2
Grapefruit-peel	(14)	87.7 ± 10.9	49.0 ± 2.7	58.7 ± 7.8
	(28)	48.0 ± 10.8	23.0 ± 4.4	55.2 ± 17.0
Mandarinpeel	(14)	86.7 ± 13.2	46.7 ± 2.0	57.0 ± 8.6
	(28)	47.3 ± 22.0	32.2 ± 17.0	65.5 ± 7.5

n = 3

[+] Percentage data were angularly transformed.

Figs. 3.3 and 3.4 show regression lines for the number of emergent adults against larvae for fixed and essential oils respectively. With the exception of essential oil trials at the lower application rate (14 ml/Kg), adult emergence was found to be significantly ($P<0.001$) correlated with larval emergence.

Fixed oils were found to reduce larval emergence at 56 and 112 ml /Kg, while essential oils gave complete control at both rates (Table 3.4). All parental adult insects in the essential oil treatments died within 48 h.

3.3.2 Secondary trials with selected fixed and essential oils

3.3.2.1 C.maculatus.

The fixed oils had no significant ($P>0.05$) effect on oviposition at either 3.5 or 14 ml/Kg (Table 3.5). However, limepeel and tangerinepeel oils at 14 ml/Kg significantly ($P<0.05$) reduced oviposition when compared with controls (Table 3.6). Two-way analysis of variance also showed significant ($P<0.001$) differences in effectiveness between the essential oils (Appendix 3.1).

The fixed oils significantly ($P<0.001$) reduced the number of emergent adults when compared with the control (Table 3.5) but showed no significant ($P>0.05$) differences between oils (Appendix 3.2). Essential oils significantly ($P<0.05$) reduced adult emergence when compared with the controls in treatments where there had been corresponding significant reduction in oviposition (Table 3.6).

Fig. 3.3 Regression analysis of adult emergence of D.maculatus against larval emergence for fixed oil treatments.

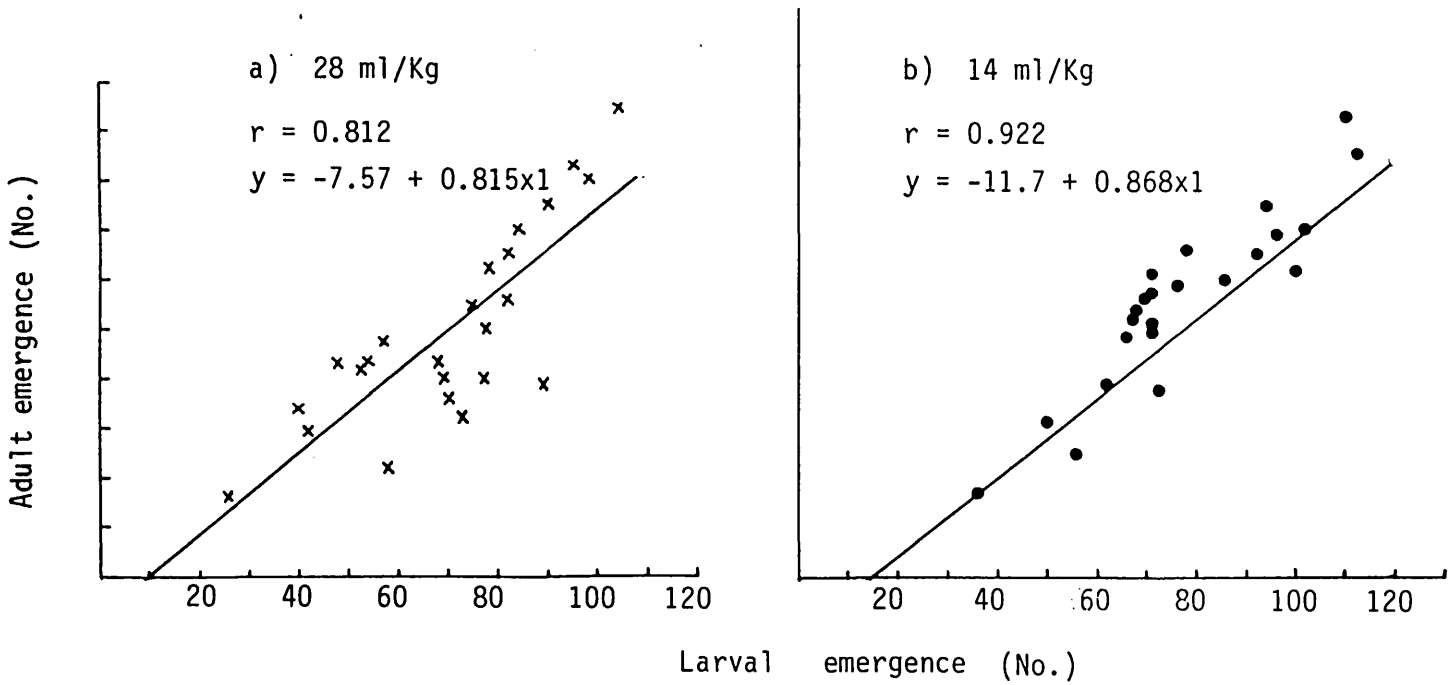


Fig. 3.4 Regression analysis of adult emergence of D.maculatus against larval emergence for essential oil treatments.

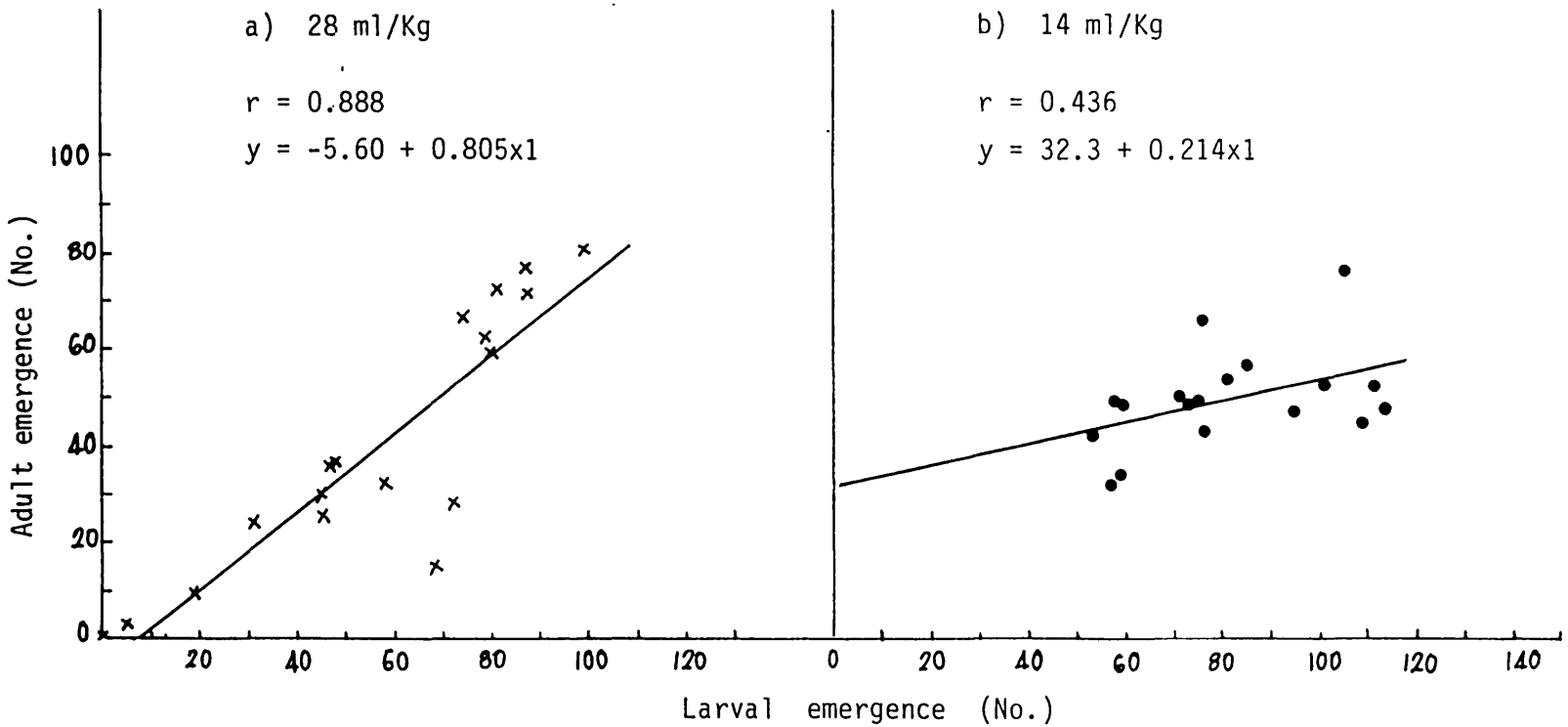


Table 3.4 Effects of fixed and essential oils on larval and adult progeny emergence of D.maculatus.

Treatments (ml/Kg)	$\bar{x}$ No. of larvae	$\bar{x}$ No. of emergent adults	$\bar{x}$ % adult emergence
Untreated Control	96.0	67.0	70.0
FIXED OILS			
Groundnut (112)	6.5	6.0	92.0
(56)	65.5	20.0	31.0
Coconut (112)	0.0	0.0	0.0
(56)	44.0	18.5	42.0
ESSENTIAL OILS			
Orangepeel (112)	0.0	0.0	0.0
(56)	0.0	0.0	0.0
Limepeel (112)	0.0	0.0	0.0
(56)	0.0	0.0	0.0

n = 2 (4 insects [2♀, 2♂] per replicate).

Table 3.5 Effects of fixed oils on oviposition and adult emergence of C.maculatus.

Treatments (ml/Kg)		$\bar{x}$ No. of eggs laid (oviposition)	$\bar{x}$ No. of emerged adults	$\bar{x}$ % adult emergence[+]
Control		126.8	84.5	67.0
Groundnut oil	(3.5)	97.5	48.7**	50.0
	(14)	95.0	0.2***	0.2
Traditional coconut oil	(3.5)	103.0	56.5**	55.0
	(14)	128.8	0.0***	0.0
Sharkliver oil	(3.5)	129.0	53.2**	41.0
	(14)	130.8	0.3***	0.2

n = 6

Pooled S.E.'s were 12.3 and 6.1 for eggs and adults respectively.

, * Significantly different from control at $P<0.01$ and $P<0.001$ respectively.

[+] Analysed further (see Table 3.7).

Table 3.6 Effects of essential oils on oviposition and adult emergence of C.maculatus.

Treatments (ml/Kg)		$\bar{x}$ No. of eggs laid (oviposition)	$\bar{x}$ No. of emerged adults	$\bar{x}$ % adult emergence[+]
Control		126.8	84.5	67.0
Limepeel oil	(3.5)	85.3	59.2	69.0
	(14)	0.0***	0.0***	0.0
Tangerinepeel oil	(3.5)	116.7	82.7	71.0
	(14)	72.0*	45.0**	63.0
Grapefruitpeel oil	(3.5)	149.0	82.0	55.0
	(14)	88.5	38.7**	44.0

n = 6

Poole S.E.'s were 12.6 and 9.2 for eggs and adults respectively.

*, **, *** Significantly different from control at $P<0.05$, $P<0.01$ $P<0.001$ respectively

[+] Analysed further (See Table 3.7)

The percentage emergence (proportion of eggs laid, survivorship into adults) data for fixed and essential oils is given in Table 3.7. The data was analysed using a Genstat Model (three-way nested ANOVA) with added controls employing substituted predicted values for all zero values which otherwise tended to distort statistical analysis. Oil treatments significantly ($P<0.001$) reduced the percentage adult emergence with significant ($P<0.001$) main interaction effects (Table 3.7, Appendix 3.3) but these effects were largely due to the fixed oils. The overall percentage adult emergence in essential oil treatments was not significantly different ($P>0.05$) from the control (Table 3.7).

3.3.2.2 S.zeamais.

The fixed oils significantly ($P<0.001$) reduced adult progeny emergence when compared with the control (Table 3.8). Two-way ANOVA showed that this effect was significantly ($P<0.001$) dosage dependent but had no significant ($P>0.05$) oil main effect (Appendix 3.4).

With essential oils, only limepeel at 14 ml/Kg and tangerinepeel at 3.5 and 14 ml/Kg significantly reduced adult emergence compared with the control (Table 3.9). Two-way ANOVA showed significant ($P<0.001$) dosage and oil main effects (Appendix 3.5).

3.3.2.3 D.maculatus.

A comparison of the treatment means for each group of oils showed that only fixed and essential oil applications at 112 and 56 ml/Kg respectively significantly ($P<0.001$) reduced emergent larvae compared with the corresponding control (Tables 3.10 and 3.11). The effects of fixed and essential oils against larval

Table 3.7 Effects of fixed and essential oils on percentage adult emergence of C.maculatus.

Treatment oils (ml/Kg)		$\bar{x}$ % adult emergence ± S.E.[+] (Type.Dose.Oil) n = 6; P<0.001	(Type.Dose) n = 18; P<0.001	(Type) n = 36; P<0.001
FIXED OILS				
Groundnut	(3.5)	50.0 ± 3.5**		
Trad. coconut	(3.5)	53.5 ± 4.4**	48.3 ± 1.8***	
Sharkliver	(3.5)	41.3 ± 3.5***		15.3 ± 3.1***
Groundnut	(14)	0.03± 0.2***		
Trad. coconut	(14)	0.0 ***	0.03± 0.06***	
Sharkliver	(14)	0.1 ± 0.3***		
ESSENTIAL OILS				
Limepeel	(3.5)	65.5 ± 3.5		
Tangerine-peel	(3.5)	73.5 ± 3.9	63.7 ± 2.5	
Grapefruit-peel	(3.5)	55.2 ± 3.5		58.7 ± 3.5
Limepeel	(14)	53.5 ± 3.5		
Tangerine-peel	(14)	62.1 ± 4.2	53.5 ± 1.8**	
Grapefruit-peel	(14)	43.0 ± 4.3		
Control		67.1 ± 3.3		

[+] Percentage data angularly transformed (predicted values substituted for zero values in computation).

, * Significantly different from control at P<0.01 and P<0.001 respectively

Table 3.8 Effects of fixed oils on adult emergence of S.zeamais.

Treatment (ml/Kg)		$\bar{x}$ adult emergence[+]
Control		69.5
Groundnut oil	(3.5)	48.2***
	(14)	3.3***
Traditional coconut oil	(3.5)	53.5**
	(14)	0.5***
Corn oil	(3.5)	48.3***
	(14)	0.2***

n = 6.

[+] Pooled S.E.'s was 3.2.

, * Significantly different from control at P<0.01 and P<0.001 respectively.

Table 3.9 Effects of essential oils on adult emergence of S.zeamais.

Treatment (ml/Kg)		$\bar{x}$ adult emergence[+]
Control		69.5
Limepeel oil	(3.5)	68.5
	(14)	15.2***
Tangerinepeel oil	(3.5)	44.0**
	(14)	39.2***
Grapefruitpeel oil	(3.5)	62.8
	(14)	60.0

n = 6

[+] Pooled S.E. was 5.1.

, * Significantly different from control at P<0.01 and P<0.001 respectively.

Table 3.10 Effects of fixed oils on larval and adult emergence of D.maculatus.

Treatment (ml/Kg)		$\bar{x}$ No. of emerged larvae	$\bar{x}$ No. of emerged adults	$\bar{x}$ % adult emergence[+]
Control		69.0	49.0	71
Groundnut oil	(28)	53.2	40.0	75
	(112)	13.0***	12.7***	98
Traditional coconut oil	(28)	63.7	42.5	67
	(112)	8.2***	7.8***	95
Sharkliver oil	(28)	75.0	48.0	64
	(112)	21.8***	16.8***	77

n = 6.

S.E.'s were 5.6 and 5.3 for larvae and adults respectively.

*** Significantly different from control at $P<0.001$

[+] Analysed further (See Table 3.12).

Table 3.11 Effects of essential oils on larval and adult emergence of D.maculatus.

Treatments (ml/Kg)		$\bar{x}$ No. of emerged larvae	$\bar{x}$ No. of emerged adults	$\bar{x}$ % adult emergence[+]
Control		69.0	49.0	71
Limepeel oil	(28)	58.8	44.8	76
	(56)	5.2***	4.3***	83
Orangepeel oil	(28)	68.2	40.7	60
	(56)	28.2***	22.3**	79
Grapefruitpeel oil	(28)	66.5	41.0	62
	(56)	25.7***	19.8***	77

n = 6.

S.E.'s were 6.9 and 5.4 for larvae and adults respectively.

, * Significantly different from control at P<0.01 and P<0.001 respectively.

[+] Analysed further (Table 3.12).

emergence were signficantly ($P<0.001$) dosage dependent (Appendices 3.6 and 3.7).

Sixty two to 98 % of emergent larvae in the oil treatments survived to become adults compared with 71 % in the control (Tables 3.10; 3.11). The data was re-analysed by a three-way nested ANOVA with added controls (see Section 3.3.2.1). The results are given in Table 3.12 and Appendix 3.8 and show a trend towards a higher percentage emergence at higher dosages although the overall effects of fixed or essential oils were not significantly ($P>0.05$) different from the controls (Table 3.12).

Tables 3.13 to 3.16 show the effects of fixed and essential oils on larval and adult body weight and the damage potential of F1 progeny during development. A comparison of treatment means showed that of the fixed oils, only traditional coconut oil and sharkliver oil at 112 ml/Kg significantly ($P>0.05$) reduced the weight of larvae and the amount of fish consumed compared with the control (Table 3.13). Likewise, of the essential oils only limepeel and grapefruitpeel at 56 ml/Kg reduced larval weight significantly ($P<0.05$; Table 3.14). Essential oils had no significant ($P>0.05$) effect on weight of fish consumed per larvae.

Only the high rate (112 ml/Kg) of fixed oils significantly increased the weights of emergent adults ($P<0.05$) compared with

Table 3.12 Effects of fixed and essential oils on percentage adult emergence of D.maculatus.

Treatment oils (ml/Kg)		$\bar{x}$ % adult emergence ± S.E. (TYPE.DOSE.OIL) n=6+ P>0.05	(TYPE.DOSE) n=18+ P<0.001	(TYPE) n=36+ P<0.05
FIXED OILS				
Groundnut	(28)	78.0 ± 5.1		
Traditional coconut	(28)	67.1 ± 6.6	70.3 ± 3.2	
Sharkliver	(28)	65.5 ± 5.8		82.1 ± 5.4
Groundnut	(112)	92.4 ± 4.6		
Traditional coconut	(112)	96.4 ± 3.3	91.5 ± 2.0*	
Sharkliver	(112)	83.5 ± 4.5		
ESSENTIAL OILS				
Limepeel	(28)	78.0 ± 5.8		
Tangerinepeel	(28)	60.4 ± 6.0	67.1 ± 3.3	
Grapefruit-peel	(28)	63.8 ± 5.9		68.7 ± 5.4
Limepeel	(56)	80.8 ± 9.0		
Tangerinepeel	(56)	80.0 ± 6.1	79.4 ± 2.8	
Grapefruit-peel	(56)	78.0 ± 6.6		
Control		70.3 ± 6.4	70.3 ± 6.4	70.3 ± 6.4

+ % data angularly transformed (predicted values substituted for zero data in computation.

* Significantly different from control (P<0.05).

Table 3.13 Effects of fixed oils on D.maculatus larval weight and larval comsumption of fish during the first 18 days of bioassay.

Treatment oils (ml/Kg)	After 18 days	
	$\bar{x}$ weight of larva (mg)	$\bar{x}$ amount of fish consumed per larva (mg)
Control	12.7	38.8
Groundnut (28)	10.3	26.8
(112)	9.3	16.3*
Traditional coconut oil (28)	16.2	40.3
(112)	4.6*	32.3
Sharkliver oil (28)	14.3	37.2
(112)	7.3*	20.2*

n = 6.

Pooled S.E.'s were 1.8 and 4.7 for larval weight and fish consumed respectively.

* Significantly different from control at $P<0.05$.

Table 3.14 Effects of essential oils on D.maculatus larval weight and larval consumption of fish during the first 18 days of bioassay.

Treatment oils (ml/Kg)		After 18 days	
		$\bar{x}$ weight of larva (mg)	x amount of fish consumed per larva (mg)
Control		12.7	39.0
Limepeel	(28)	10.5	33.8
	(56)	5.0*	23.0
Tangerine-peel	(28)	8.3	30.0
	(56)	8.5	32.0
Grapefruit-peel	(28)	8.5	29.0
	(56)	6.0*	30.0

n = 6.

Pooled S.E.'s were 1.9 and 3.2 for larval weight and fish consumed respectively.

* Significantly different from control at $P<0.05$.

Table 3.15 Effects of fixed oils on D.maculatus adult weight and the amount of fish consumed (damage) by total F1 progeny during development.

Treatment oils (ml/Kg)	$\bar{x}$ weight of emergent adult (mg) P<0.001	$\bar{x}$ weight of fish consumed per successful F1 adult (mg)++ P<0.05	$\bar{x}$ weight of fish consumed by total F1 progeny (g) + P<0.001
Control	26.7	252.0	11.9
Groundnut (28)	28.3	244.0	9.8
(112)	39.0*	179.0	3.5***
Tradition-al coconut (28)	27.8	277.0	10.9
(112)	38.3*	157.0*	1.8***
Shark-liver (28)	27.8	269.0	12.1
(112)	35.0*	202.0	3.9***

n = 6.

Pooled S.E.'s were 2.0, 26.0, 0.87 for adult weight, weight of fish consumed per F1 adult and fish consumed by total F1 progeny respectively.

*, *** Significantly different from control at P<0.05 and P<0.001 respectively.

+ Fish consumed out of 15g per replicate.

++ $\frac{\text{Total weight of fish consumed by F1 progeny}}{\text{Total number of F1 adults}}$

Table 3.16 Effects of essential oils on D.maculatus adult weight and the amount of fish consumed (damage) by total F1 progeny during development.

Treatment oils (ml/Kg)	$\bar{x}$ weight of emergent adult (mg) P<0.001	$\bar{x}$ weight of fish consumed per successful F1 adult (mg)++ P<0.05	$\bar{x}$ weight of fish consumed by total F1 progeny (g) + P<0.001
Control	26.7	252.0	11.85
Limepeel (28)	27.2	234.0	10.40
(56)	41.0***	421.0***	3.57***
Tangerine-peel (28)	28.0	297.0	11.67
(56)	27.8	250.0	8.40*
Grapefruit-peel (28)	25.3	255.0	10.38
(56)	27.8	224.0	6.70***

n = 6.

Pooled S.E.'s were 1.5, 25 and 0.93 for adult weight, fish consumed per F1 adult and fish consumed by total F1 progeny respectively.

*, *** Significantly different from control at P<0.05 and P<0.001 respectively.

+ Fish consumed out of 15g per replicate.

++ $\frac{\text{Total weight of fish consumed by F1 progeny}}{\text{Total number of F1 adults}}$

controls (Table 3.15). With essential oils, only limepeel oil at 56 ml/Kg significnatly ($P<0.001$) affected the weight of emergent adults; adults in this treatment being larger than controls (Table 3.16). This rate of limepeel oil also significantly increased the weight of fish consumed per successful adult progeny compared with controls ($P<0.001$; Table 3.16).

All fixed and essential oils applied at 112 and 56 ml/Kg respectively significantly ($P<0.05$) reduced the amount of fish consumed by developing F1 progeny (total damage) compared with corresponding controls (Tables 3.15 and 3.16); only fish in these high dosage treatments having been protected from significant damage.

3.3.3 Rate of loss of biological activity

3.3.3.1 Relative rate of evaporation of fixed and essential oils. Seventy % by weight of limepeel oil was lost within 6 h of exposure at 28 °C; after the 7th day of exposure the weight of oil remained constant (Fig. 3.5). All the essential oils tested showed a similar rapid rate of evaporation when tested at room temperature (Fig. 3.6). In contrast, groundnut oil exposed at 28° C lost no weight over 15 days. Similarly, all other fixed oils tested showed no evaporation from filter paper surfaces over 24 h at room temperature.

In a follow up experiment it was shown that the rate of evaporation of limepeel oil placed inside a closed Petri dish or honey jar was reduced to varying degrees (Fig. 3.5). These differences in rates of evaporation are reflected in the effect

Fig. 3.5 Percentage weight loss of limepeel oil at 28°C.

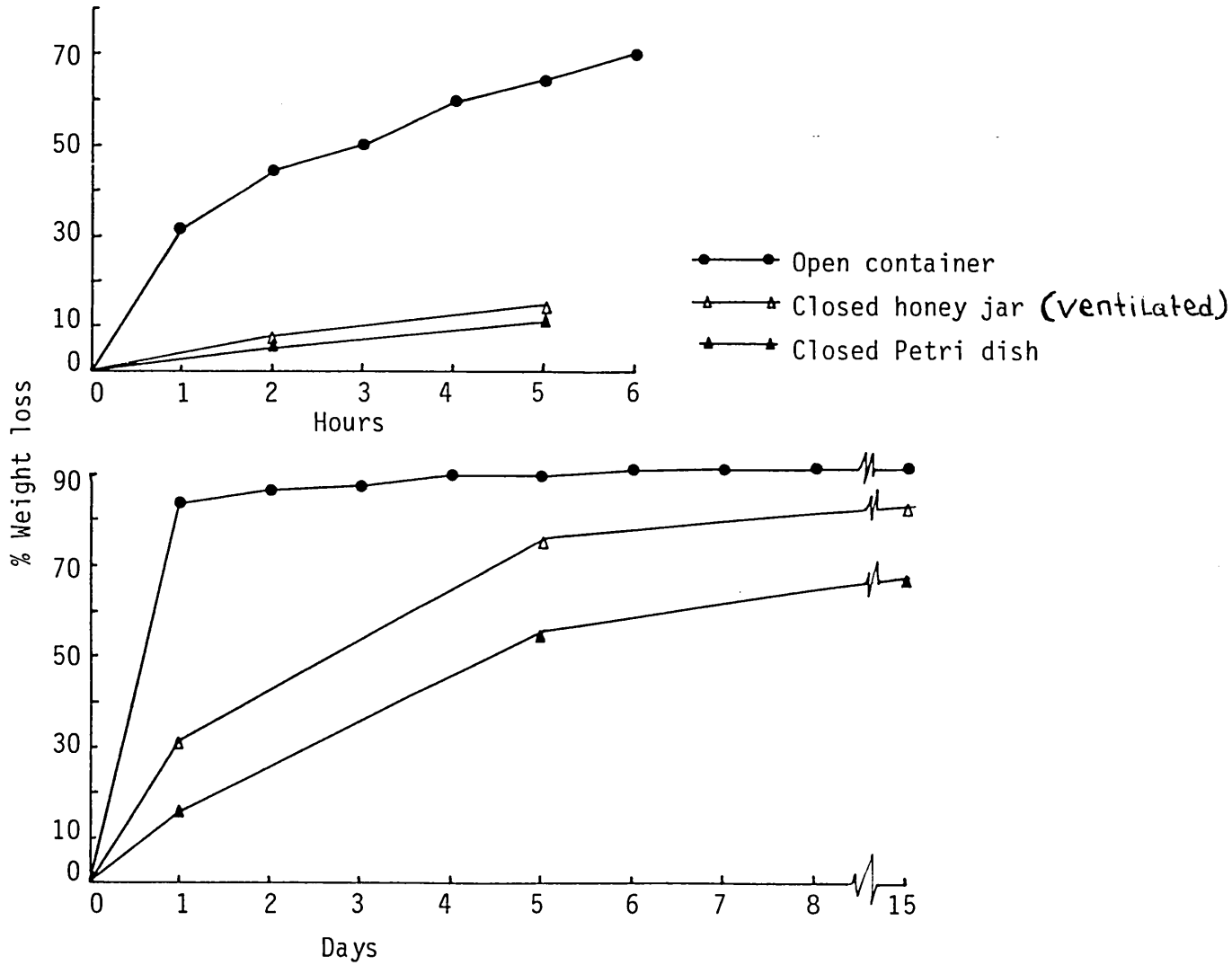
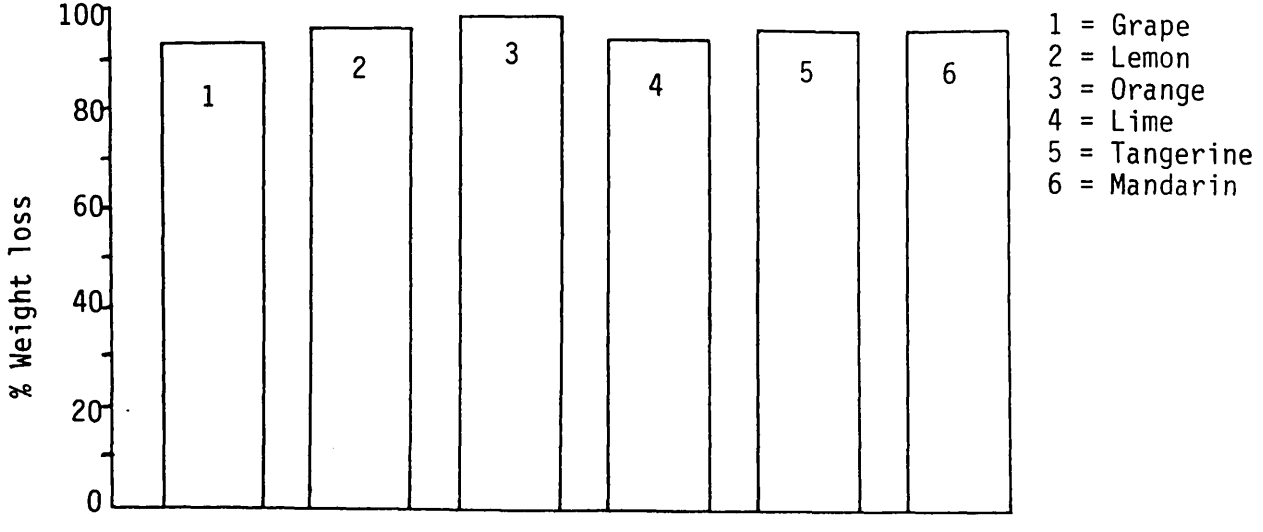


Fig. 3.6 Percentage weight loss of essential oils over 6 h at room temperature.



of limepeel oil on adult mortality and oviposition in Petri dishes and honey jars (Table 3.17). Groundnut oil had Little effect on adult mortality and oviposition in both types of containers (Table 3.17).

3.3.3.2 Effect of the time lag between oil application and bioassay.

In bioassays which commenced 1 h after application of limepeel oil to cowpea grains there was 100 % adult mortality for C.maculatus and no progeny development. In contrast, when the bioassays commenced 24 h after application of oil there was no appreciable adult mortality and progeny development was comparable with the control (Table 3.18). Limepeel oil on fish strips showed a similar loss in activity against D.maculatus (Table 3.19).

With groundnut oil there was no differential effects on adult mortality or progeny development when bioassays commenced 1 or 24 h after application (Table 3.18).

3.4 Discussion

Effects of fixed and essential oils on progeny development.

In laboratory bioassays many workers have shown that plant oils can limit progeny development of stored product insects, particularly in bruchid and Sitophilus beetles (Mital, 1971; Su et al., 1972b; Mummigatti and Ragunathan, 1977; Schoonhoven, 1978; Singh et al., 1978; Mensina and Renwick, 1983). However, there appear to have been no comparative studies on the biological

Table 3.17 Effect of container on activity of oils against C.maculatus.

Treatment oils[+] (container)	$\bar{x}$ % parental adult mortality after 24h (out of 60)	$\bar{x}$ number of eggs laid
Control (Petri dish)	1	72
(Honey jar)	2	61
Groundnut (Petri dish)	3	66
(Honey jar)	3	78
Limepeel (Petri dish)	100	0
(Honey jar)	63	10

[+] Oils applied at 14ml/Kg.

n = 3 (20 insects per replicate).

Table 3.18 Effect of the time interval between oil application and introduction of insects on the toxicity of oils against C.maculatus.

Treatment (Time interval in h)	$\bar{x}$ mortality of parental adult (out of 20) at 24h	$\bar{x}$ eggs laid	$\bar{x}$ adult emergence	$\bar{x}$ % adult emergence
Control	0	128.6	89.6	69.7
Groundnut oil+(1)	0	45.6	36.0	79.0
(24)	0	38.0	35.0	92.0
Limepeel oil+ (1)	20	0.0	0.0	0.0
(24)	1	84.0	46.0	55.0

+ Oils applied at 7ml/Kg.

n = 5 (4 insects per replicate).

Table 3.19 Effect of the time interval between oil application and introduction of insects on the toxicity of limepeel oil against D.maculatus.

Treatment (Time interval in h).	$\bar{x}$ mortality of adults (out of 60) at 48h	$\bar{x}$ emergent larvae
Control	0	145
Limepeel oil+(1)	59	3
(24)	23	108
(240)	0	155

+ Oil applied at 28ml/Kg.

n = 3 (20 insects [mixed sexes] per replicate).

effects on insects of groups of plant oils which have different physio-chemical properties.

In this study, the results show that a distinction can be made between the way fixed and essential oils affect insects. A clearer understanding of these differential mechanisms of action could lead to more efficient use of such oils for insect control.

Oviposition and adult emergence of C.maculatus.

In the present work, low and high rates of fixed oils were found to have no significant effect on oviposition. This contradicts most reports in the literature (see Singh et al., 1978; Hill and Schoonhoven, 1981). For example, Schoonhoven (1978) reported that palm and cotton seed oils significantly reduced total egg production of the bruchid Zabrotes subfasciatus. While, Mensina and Renwick (1983) found that peanut, coconut and safflower oils deterred oviposition of C.maculatus at the low rate of 5 ml/Kg although these authors mentioned that this effect was not particularly strong for seeds tested 1 day after oil application.

Nwanze et al. (1975) have shown that egg laying females of C.maculatus can detect macroscopic differences in seed coat texture. This suggests that these beetles should be capable of detecting the presence of oil on seed coats particularly at high doses. It therefore seems likely that the insects percieved the oil films on seeds but went on to lay eggs in a no-choice situation. This was possibly because the fixed oils had no direct toxic effect on adult C.maculatus, an observation confirmed by other experiments in the present study (Section 4). The

possibility of oil affecting the pattern of oviposition and preferences in a choice situation is examined later.

In contrast, essential oils were found to significantly reduce oviposition of C.maculatus in two successive experiments. Observations showed that where adult mortality occurred few eggs or none at all were laid. Whereas in treatments which caused no adult mortality, oviposition appeared to be normal. This suggested that essential oils reduced oviposition by causing rapid adult mortality; citrus peel oils having been shown to have strong contact toxicity against C.maculatus adults (Su et al., 1972a).

In the present work, although fixed oils did not deter oviposition by C.maculatus they did significantly reduce the subsequent emergence of adults. This is in agreement with reports on the action of vegetable oils on insects (see Schoonhoven, 1978; Periera, 1983).

In the second set of experiments it was shown that fixed oils significantly reduced the percentage adult emergence based on total number of eggs laid. These results implied that fixed oils reduced the number of adult progeny by acting against eggs and/or larvae. Mensina and Renwick (1983) have also reported that vegetable oils (at 5 ml/Kg) effectively protected cowpeas from C.maculatus by reducing adult emergence. These authors concluded that the oils caused high mortality of eggs and larvae on the seed surface. Similarly, Singh et al. (1978) showed that groundnut oil treatments prevented emergence of C.maculatus adult progeny.

Essential oils also significantly reduced adult emergence in C.maculatus. This is in agreement with the work of Su et al. (1972b) who showed that peel oils from 8 citrus fruits protected cowpeas from C.maculatus infestation by reducing oviposition and adult emergence. However, the present study has shown that a significant reduction in adult emergence only occurred in treatments where there had been a corresponding reduction in oviposition. In these experiments, it was shown that percentage adult emergence in essential oil treatments was not significantly different from the control. Thus, essential oils appear to act by reducing oviposition through adult mortality but have little or no residual activity on eggs or larvae. This suggestion appears to be in conflict with the work of Su et al. (1972b) who showed that the insecticidal activity of the citrus oils they tested persisted for over 300 days. However, these authors applied the non-volatile fraction of citrus oils at 1 % by weight of cowpeas. In this case the oil may have remained in the seed coat and continued to act against eggs in a similar way to fixed oils.

Adult progeny emergence of S.zeamais.

All the fixed oils tested significantly reduced adult progeny emergence. The effects of these oils on oviposition were not assessed in these experiments but subsequent X-ray radiographic studies (Section 4) suggested a similar effect to that with C.maculatus; the major action being on egg or larval development rather than oviposition. There are few other reports on the action of plant oils against Sitophilus species. However, a recent paper by Qi and Burkholder (1981) has also shown that four

vegetable oils protected wheat from the granary weevil, S.granarius by reducing adult emergence.

The essential oils tested were also found to be effective in reducing adult progeny emergence although this action was usually restricted to higher rates of application. Observations during oviposition showed that F1 adult progeny emergence was only significantly reduced in treatments where essential oils caused mortality of parental adults within the first 48 h of the bioassay. The toxic action of essential oils against the adults was confirmed in later experiments (Section 5).

Thus, as in C.maculatus the principal action of essential oils against S.zeamais was on oviposition. The apparent rapid loss of activity of essential oil treatments was attributed to the evaporation of volatile components from the grain surface in Petri dish experiments. This may also explain the moderate to insignificant activity of essential oils assayed on grains 18 h after application.

Larval and adult progeny emergence of D.maculatus.

Preliminary experiments indicated that fixed oils at 14 and 28 ml/Kg had no apparent effect against larval emergence and adult progeny development. Dr. C. Wood (personal communication) has also observed that application of a film of groundnut oil (rate unknown) to dried fish failed to prevent damage by D.maculatus progeny. However, at a higher rate (112 ml/Kg) fixed oils were found to effectively reduce progeny development and were thus able to protect dried fish.

In a survey of dried fish markets in Nigeria it was found (K.N. Don-Pedro, unpublished observations) that traditional dried fishmongers have been applying generous amounts of plant oils on fish in the belief that this renders the produce more attractive to buyers and reduces pest damage. Such amounts may be equivalent to the highest rate tested.

In the present study it was observed that fixed oils did not kill adults except by "drowning", which was rare, and thus the significant reduction in progeny development could have been due to effects on oviposition or on the eggs. It is perhaps less likely to be oviposition because the oils did not normally affect adults and have been shown to be ineffective against oviposition with C.maculatus and S.zeamais (see Section 4). Activity against eggs was more probable and this was examined in later experiments.

Compared with grains, relatively large amounts of oil per unit weight of stored product were needed to achieve insect control on fish. This may have been due to the fish muscle being more absorbent, so that at lower rates of application there was little or no surface oil film available to act against eggs. Alternatively, fixed oils may be intrinsically less active against D.maculatus than against C.maculatus or S.zeamais. These possibilities were investigated in later experiments (Section 4.2).

Essential oils significantly reduced the number of emergent larvae only at the highest rate tested of 56 ml/Kg. As in the case of C.maculatus and S.zeamais, essential oils appeared to act

on larval emergence by causing rapid mortality of parental adults (see also Section 5).

Both fixed and essential oils significantly reduced the number of emergent adults of D.maculatus but this was related to the number of emergent larvae and no toxic effects on F1 adults were observed. In fact, fixed oils at 112 ml/Kg significantly increased the percentage adult emergence. This was probably due to the reduced number of larvae that emerged in such treatments being given a better chance of survival due to less competition and cannibalism. For example, Osuji (1973) in his study on the biology of D.maculatus reported some larval cannibalism under crowded conditions.

After the first 18 days of larval emergence it was shown that traditional coconut and sharkliver oils applied at 112 ml/Kg significantly reduced larval weight and the amount of fish consumed per larva. While of the essential oils, limepeel and grapefruitpeel oils at 56 ml/Kg significantly reduced larval weight but had no effect on the amount of fish consumed per larva.

The above results indicate that generous application of some plant oils can potentially deter larval feeding during the early stages following emergence. This may also account for reduced larval weights found in such treatments. The observed reduction in larval weights was probably due more to the delayed hatching of eggs that survived oil treatments. This is supported by observations which showed that development into adults generally proceeded at a slower rate (based on data of earliest adult

emergence) in all effective oil treatments; development taking 2-3 days longer than in the controls.

However, the observed reductions in larval weight in some oil treatments appear to have been a temporary feature because the weights of adults that developed from the larvae treated with high rates of fixed and essential oils were significantly greater than in the controls. In agreement with these results was the observation that oil treatments did not significantly alter the amount of fish consumed per successful adult progeny.

Thus, neither fixed nor essential oils had long-term antifeedant activity against D.maculatus. In the case of essential oils, if such properties exist it is likely that they are due to the loss of active volatile components from the fish surface. The observed protection of dried fish (based on weight of fish consumed) by all the fixed and essential oils when applied at rates effective against the insect was due to reduction in numbers of emergent progeny rather than to effects on feeding.

Rate of loss of biological activity

All the essential oils tested lost over 90% by weight through evaporation from filter paper in 6 h at room temperature in an open laboratory. The rate of evaporation of limepeel oil was slightly reduced in the Petri dishes and ventilated honey jars used in the bioassays. Further work has shown that it is the volatile fraction of citrus oils which is toxic to insects

(Section 5) and the variations in activity of limepeel oil found against C.maculatus in honey jars and Petri dishes was most probably due to different rates of diffusion of volatile components from the surfaces of treated grains or dried fish.

These observations would also explain why the activity of limepeel oil against C.maculatus and D.maculatus was found to be dependent on the time interval between the application of the oil and commencement of the bioassay. It was therefore evident that a better way to use essential oils to control insects would be as a fumigant in a relatively enclosed or airtight system.

In contrast, groundnut oil (which like all other fixed oils does not have a volatile component) showed an activity against the test insects which was independent of the type of bioassay container used and the time interval between application and bioassay. Vegetable oils including groundnut oil have in fact been reported to protect grains against C.maculatus progeny development for up to 180 days (Singh et al., 1978).

MECHANISM OF ACTION OF FIXED OILS AGAINST INSECTS

4.1 Introduction.

In the preceeding chapter, it was established that the application of both vegetable fixed oils and citrus essential oils could effectively reduce F1 progeny development of C.maculatus on cowpea, S.zeamais on wheat and D.maculatus on dried fish. Fixed and essential oils were also shown to differ in their mode of action although specific actions of the former remained unclear.

In this section, the stages in the life cycle of test insects which were susceptible to fixed oils at rates comparable with those that gave effective progeny control were examined. On the basis of the fatty acid composition of vegetable oils (Sheppard et al., 1978), the three major components - lauric, oleic and linoleic acids (See Table 1.2) were also tested against C.maculatus in an attempt to determine the principal active ingredient.

4.2 Materials and Methods.

4.2.1 Direct toxicity of fixed oils against adults

4.2.1.1 C.maculatus.

Groundnut and traditional coconut oils were applied to cowpea grains at 3.5, 7.0 and 14 ml/Kg. Twenty, 1-2 day-old adults (mixed sexes) were confined with 20 treated or untreated grains 1 h after oil application. A total of 40 insects were tested per

treatment. Mortality was assessed after 24, 48 and 120 h.

4.2.1.2 S.zeamais.

Twenty, 2-12 day-old adults (mixed sexes) were confined with 10 g (>64 grains) of treated or untreated wheat grains in a Petri dish (7 cm dia.). Bioassay commenced 1 h after application of oil (3.5, 14 and 17.5 ml/Kg). Each treatment was replicated 4 times. Mortality was assessed after 24, 48 and 158 h.

In a second, similar experiment, bioassay commenced 14 days after oil application at 7, 14 and 17.5 ml/Kg. After assessment of adult mortality at 7 days, adults were removed and the grains were incubated in separate vials to determine possible effects on adult emergence (Section 2.2.3.3).

4.2.1.3 D.maculatus.

Groundnut and traditional coconut oils were topically applied (Section 2.2.3.3) to the dorsal prothoracic region of 1-15 day-old adults (mixed sexes). Insects were dosed at 1.0 and 2.0 µl per insect. Control insects were similarly handled but not treated. Thirty insects were tested per treatment. Mortality was assessed after 24, 48 and 120 h.

In a second experiment, one drop (approx. 0.03 ml) of groundnut or traditional coconut oil was brushed over the surface of a glass Petri dish (7 cm dia.) to give a film of oil. About 1 h after application, ten 1-15 day-old adults (mixed sexes) were confined to the treated surfaces of the Petri dish. Thirty insects (10 per dish) were tested per treatment. Mortality was assessed at 24, 48 and 120 h.

4.2.2 Effects of fixed oils on oviposition

4.2.2.1 C.maculatus: No-choice experiment (daily oviposition). Groundnut and traditional coconut oil were applied to cowpea grains at 3.5 and 14 ml/Kg (Section 2.2.1.4) about 1 h before bioassay commenced on the first day. Ten treated or untreated grains were placed in a glass Petri dish (7 cm dia.), with seven sets of Petri dishes per treatment (each set to hold ovipositing insects for 24 h). Each treatment per day was replicated 6 times.

Two, 0-24 hour-old adult insects (1♀, 1♂) were released into each of the first set of Petri dishes containing treated or untreated grains. After 24 h, the insects were transferred to the second set of Petri dishes containing treated or untreated grains and the process repeated for 7 days. Daily egg counts were made separately for each individual mated female.

4.2.2.2 C.maculatus: Two-way choice experiment (daily oviposition).

The choice chamber consisted of a 7 cm dia. glass Petri dish with 5 untreated cowpea grains placed in one half and 5 treated grains in the other. Care was taken to select grains of equal size since size is known to influence oviposition preference in C.maculatus (Nwanze et al., 1975). For both groundnut and traditional coconut oils, experiments were run for seven days and seven sets of choice chambers per treatment per day were set up at the same time as follows:

1. Untreated vs 14 ml/Kg (6 replicates per day).

2. Untreated vs 3.5 ml/Kg (6 replicates per day).
3. Untreated vs untreated (2 replicates per day).
4. 14 ml/Kg vs 14 ml/Kg (1 replicate per day).
5. 3.5 ml/Kg vs 3.5 ml/Kg (1 replicate per day).

Two, 0-20 hour-old insects (1♀, 1♂) were added to each of the first set of choice chambers 2 h after oil application. Insects were transferred to new chambers every 24 h for 7 days. The total number of eggs laid on each batch of 5 grains was assessed separately (Section 2.2.3.3).

4.2.2.3 S.zeamais: No-choice experiment.
Groundnut, traditional coconut, corn and sesame oils were applied to wheat grains at 3.5 and 14 ml/Kg. About 5 h after oil application, six, 8-15 day-old adults (3♀, 3♂) were confined for 11 days with 5 g of treated or untreated grains in Petri dishes. All treatments were replicated 3 times.

X-ray radiographs of each batch of grains were taken at 16 and 29 days after commencement of bioassay (the latter a few days before F1 emergence). By examining X-ray films (Section 2.2.3.3) the number of eggs laid was assessed.

4.2.2.4 S.zeamais: Two-way choice experiment.
In this experiment only groundnut oil was examined; the choice chamber (Section 4.2.2.2) containing 40 untreated grains in one half and 40 treated grains in the other. One set of choice chambers were used per treatment and four, 14-20 day-old adults (2♀, 2♂) were added to each dish 2 h after oil application. Daily oviposition was not measured as preliminary experiments indicated

that when exposed to new sets of grains S.zeamais females failed to lay eggs for the first few days. The treatments were as follows:

1. Untreated vs 14 ml/Kg (5 replicates).
2. Untreated vs 3.5 ml/Kg (5 replicates).
3. Untreated vs untreated (1 replicate).
4. 14 ml/Kg vs 14ml/Kg (1 replicate).
5. 3.5 ml/Kg vs 3.5 ml/Kg (1 replicate).

After 7 days, adults were removed and each batch of 40 grains arranged in plastic frames for X-ray radiography. Radiographs were taken at 13 and 29 days after commencement of the bioassay and the total number of eggs laid per batch of grains assessed

4.2.3 Ovicidal activity of fixed oils and their major fatty acid components against eggs

4.2.3.1 C.maculatus.

a. Pre-infestation grain treatment. Two experiments were carried out in which insects were allowed to oviposit on grains pre-treated with 1) groundnut or traditional coconut oil; 2) lauric*, oleic or linoleic acids. Application rates ranged from 1.4 to 14 ml/Kg. In the experiment with oils, six 1-2 day-old adults (3♀, 3♂) were confined in Petri dishes with treated or untreated seeds (5 replicates per treatment) for 24 h; grains were then transferred to ventilated vials. With the component fatty acids, four 1-3 day-old adults (2♀, 2♂) were confined for 5 days in Petri dishes with treated or untreated grains (4 replicates per treatment). In both experiments, mortality was

* 10 % (w/v) in acetone [0.823 g lauric acid (equivalent wt to 1 ml groundnut oil) made up to 10 ml with acetone].

assessed 12 days after oviposition (Section 2.2.3.3).

b. Post-infestation grain treatment. One to two day-old eggs were treated by dipping (Section 2.2.3.3) infested grains in acetone-based solutions of groundnut oil, traditional coconut oil, lauric acid, oleic acid or linoleic acid (3.2 - 50 % v/v). Each treatment had a variable number of eggs in a total of 15 - 20 grains (5 grains per replicate). Grains were incubated in ventilated vials. Egg mortality was assessed after 12 days and subsequent adult emergence was also recorded (Section 2.2.3.3).

4.2.3.2 D.maculatus.

Dried fish strips (10 g) were treated with groundnut oil at rates between 14 and 56 ml/Kg. Thirty 0-2 day-old eggs (15 eggs per replicate) were added 2 h after oil application. Mortality was assessed indirectly by counting the number of emergent larvae after 10 days (Section 2.2.3.3).

4.2.4 Mode of action of fixed oils against C.maculatus eggs

4.2.4.1 Respiratory activity.

In the first experiment, the CO_2 emission of newly laid eggs (0 - 15 hour-old) on pre-treated cowpea grains (groundnut oil at 14 ml/Kg) and on untreated grains was compared continusously for 6 days. Infested grains were incubated at 27° C and 70 % RH in chambers connected to a 6 channel CO_2 analyser, model ADC225 Mk3.

In a second experiment, eggs (0 - 15 h) on grains were dipped in 25 % (v/v) groundnut oil in acetone; CO_2 emission being measured before and after oil application.

In both the above experiments the CO_2 emission from a 20 g batch of uninfested cowpea grains was also monitored.

4.2.4.2 Action on embryonic stages.

Eggs were laid over 24 h by 10 pairs of 1-2 day-old adults on 20 g batches of treated (groundnut or traditional coconut oil at 14 ml/Kg) or untreated cowpeas. All treatments were replicated 4 times. Fourteen days after oviposition, the total number of eggs laid, the total number of unhatched eggs, and the number which had died at the late embryo stage (1st larval instar) were recorded.

4.2.4.3 Effects of solvent on embryonic development.

One to 2 day-old eggs laid on cowpea grains were dipped in various concentrations of groundnut oil in acetone or in 100% groundnut oil. After 14 days the number of unhatched eggs that had died at the late embryo stage was recorded.

4.2.4.4 Effects on hatching.

A Sellotape membrane, mounted smoothly on a microscope slide was treated with groundnut oil to form a thin film and then placed in a Petri dish with twenty 0-1 day-old adults (10♀, 10♂) for 3 days. Each treatment was replicated twice. After 20 days the number of eggs which had developed to the late embryo stage was recorded. In addition, by carefully peeling off the Sellotape membrane the number of 1st instar larvae that had successfully hatched by boring visible holes through the membrane could be counted.

4.2.4.5 Effects on attachment of eggs to grains.
Ten grains with a known number of eggs (4 - 5 day-old) from groundnut oil-treated (14 ml/Kg) or untreated batches of cowpeas were each transferred to a glass jar. The jar was then shaken on a Griffin flask shaker (setting 7; Section 2.2.1.3) for consecutive 2, 4 and 8 minute intervals. After each shaking period, the number of eggs remaining on the grains was counted. The experiment was repeated 3 times.

4.2.5 Effects of fixed oils and their major fatty acid components on progeny development

4.2.5.1 C.maculatus: Pre and post-oviposition treatment.
In the experiments described in section 4.2.3.1, grains were incubated in ventilated vials and adult emergence assessed together with oviposition and egg mortality. From this data the number of progeny that died after penetrating seeds was subsequently calculated. In addition, in experiments involving the pre-oviposition application of fatty acids, parental adult mortality was assessed at the end of the oviposition period.

4.2.5.2 C.maculatus: (10 - 11 day-old larvae).
Six to eight cowpea grains pre-infested with a total of 30 - 32 larvae were mixed with uninfested grains to give a 15 g batch. Groundnut and traditional coconut oils (1.75 - 14 ml/Kg) were applied by shaking (Section 2.2.1.4; the shaking time being reduced to 5 min to prevent damage to larvae). The untreated control was also shaken for 5 min. All treatments were replicated 4 times. Treated and untreated grains were kept in ventilated vials and total adult emergence recorded over 40 days (Section

2.2.3.3).

In a second experiment, the above oils were tested at 17.5 ml/Kg

4.2.5.3 C.maculatus: (20-21 day-old larvae/pupae).
Late stage larvae/pupae (within grains) were tested with groundnut and traditional coconut oils (3.5 - 17.5 ml/Kg) as described in section 4.2.5.2.

4.2.5.4 Penetration of oil into cowpea grain.
The penetration of fixed oils into cowpea grains was investigated using a bioassay technique. Cowpea grains were treated with groundnut oil at 3.5 - 14 ml/Kg and 1 and 10 days after oil application 3 batches of 5 seeds per treatment were assayed as:

1. Intact seeds.
2. With the seed coat peeled off.
3. With the seed coat and top layer of the endosperm removed.

Two, 1 - 2 day-old adult C.maculatus (2♀, 2♂) were confined with each batch of grains for 2 days. All treatments were replicated 4 times. Twelve days after the removal of parental adults, the total number of eggs laid and egg mortality was assessed.

4.2.5.5 S.zeamais: (pre-oviposition treatment).
From the experiment described in section 4.2.2.3, the X-ray radiographs were examined for additional data on number of early stage larvae and pupae inside treated and untreated grains. No attempt was made to determine the actual larval instars present. This was because the two X-ray radiographs taken did not provide

enough information to permit accurate determination of changes in larval head size and/or distances moved from egg deposition sites on which instar identification depended (B. Thind, personal communication). Adult emergence was also assessed for 60 days following oviposition.

4.2.5.6 S.zeamais: (post-infestation treatment, 3 - 14 day-old eggs/larvae).

Groundnut or traditional coconut oil was applied (Section 2.2.1.4) at 3.5 -14 ml/Kg to batches of pre-infested wheat grains (10 g) with immature stages (eggs and early larval stages). Each treatment was replicated 3 times.

A similar experiment was carried out with older larvae/pupae (16 - 27 day-old).

4.2.5.7 D.maculatus: (topical and glass film application).

a. Groundnut and traditional coconut oils were topically applied to larvae (22 - 24 day-old) at 1 or 2 µl per insect. Thirty larvae were used per treatment.

   Mortality was assessed after 24, 48 and 120 h.

b. Thirty µl of groundnut or traditional coconut oil was brushed over the surface of a glass Petri dish (7 cm dia.). Batches of 15, 10 - 11 or 22 - 24 day-old larvae were transferred to each dish. Thirty larvae

were taken per treatment (2 replicates of 15 insects each).

Mortality was assessed at 24, 48 and 120 h.

4.3 Results.

4.3.1 Direct toxicity of fixed oils against adults

4.3.1.1 C.maculatus.

Groundnut and traditional coconut oils applied to cowpea grains caused no apparent increase in adult mortality compared with controls after 158 h (Table 4.1).

4.3.1.2 S.zeamais.

Grains treated with groundnut and traditional coconut oil and bioassayed 1 h after application gave no appreciable adult mortality after 48 h but some mortality was found with the highest dosages of either oil after 158 h (Table 4.2).

Grains bioassayed 14 days after application caused no mortality after 48 h and only slight mortality after 158h (Table 4.2). However, the grains bioassayed 14 days after oil treatments still caused a significant reduction ($P<0.001$) in F1 adult emergence compared with controls (Table 4.3).

4.3.1.3 D.maculatus.

Topical application of groundnut oil or traditional coconut oil at 1 and 2 µl per insect had no effect on adult mortality compared with the control after 120 h (Table 4.4). Similarly, neither oil was toxic to adults after exposure to oil-treated glass surfaces for 120 h (Table 4.5).

4.3.2 Effects of fixed oils on oviposition

Table 4.1 Mortality of adult C.maculatus exposed to gorundnut oil and traditional coconut oil on cowpea grains.

Treatments	(ml/Kg)	No. dead[+] (%) 48h	158h
Control		1 (3)	12 (30)
Groundnut oil	(3.5)	1 (3)	6 (15)
	(7)	0 (0)	10 (25)
	(14)	3 (8)	13 (33)
Traditional coconut oil	(3.5)	3 (8)	5 (13)
	(7)	1 (3)	4 (10)
	(14)	2 (5)	8 (20)

[+] n = 40.

Table 4.2 Mortality of S.zeamais adults exposed to oil treated wheat 1h (a) and 14 days (b) after oil application.

Treatments (ml/Kg)		No. dead[+] (%) 48h	158h
a			
Control		0 (0)	1 (1.3)
Groundnut oil	(3.5)	0 (0)	2 (2.5)
	(14)	3 (3.8)	12 (15.0)
	(17.5)	6 (7.5)	30 (37.5)
Traditional coconut oil	(3.5)	0 (0)	0 (0)
	(14)	0 (0)	15 (18.8)
	(17.5)	0 (0)	37 (46.3)
b			
Groundnut oil	(7)	0 (0)	0 (0)
	(14)	0 (0)	0 (0)
	(17.5)	0 (0)	1 (1.3)
Traditional coconut oil	(7)	0 (0)	0 (0)
	(14)	0 (0)	2 (2.5)
	(17.5)	0 (0)	10 (12.5)
Control		0 (0)	0 (0)

[+] n = 80

[a] Bioassay 1h after treatment

[b] Bioassay 14 days after treatment

Table 4.3 Effects of groundnut and traditional coconut oil treated grains aged for 14 days on adult emergence of S.zeamais.

Treatments (ml/Kg)		$\bar{x}$ adult emergence ± S.E. n = 4 P<0.001
Control		51.8 ± 2.9
Groundnut oil	(7)	30.0 ± 2.9
	(14)	8.0 ± 2.9
	(17.5)	4.5 ± 2.9
Traditional coconut oil	(7)	23.3 ± 2.9
	(14)	4.0 ± 2.9
	(17.5)	0.5 ± 2.9

All oil treatments were significantly different from control at P<0.001.

Table 4.4 Mortality of adult D.maculatus topically treated with groundnut and traditional coconut oil.

Treatments (µl/insect)		No. dead + (%) 24h	48h	120h
Control		0	1 (3)	1 (3)
Groundnut oil	(1)	0	2 (7)	2 (7)
	(2)	0	1 (3)	1 (3)
Traditional coconut oil	(1)	0	0	0
	(2)	1 (3)	1 (3)	1 (3)

+ n = 30

Table 4.5 Mortality of adult D.maculatus exposed to groundnut and traditional coconut oil treated glass surfaces.

Treatment	No. dead + (%) 24h	48h	120h
Control	0	0	1 (3)
Groundnut oil*	0	1 (3)	1 (3)
Traditional coconut oil*	0	0	0

+ n = 30

* Approximately 0.03ml/38.5cm^2

4.3.2.1 C.maculatus: No-choice experiment (daily oviposition).
The number of eggs laid on oil-treated grains was significantly ($P<0.01$) less than controls on the first day, but between day 2 and 7 there was no significant ($P>0.05$) difference between oil treatments and controls. Overall, oil treatments had no significant ($P>0.05$) effect on the total number of eggs laid by females over the seven day test period (Fig. 4.1; Appendix 4.1).

4.3.2.2 C.maculatus: Two-way choice experiment (daily oviposition).
Adult females were not deterred from laying eggs on grains treated with groundnut oil at 3.5 ml/Kg on the first day but were on subsequent 6 days. All other treatments of groundnut oil and all treatments with traditional coconut oil significantly ($P<0.05$) reduced oviposition on treated grains from day 1 to 7 (Fig. 4.2; Appendix 4.2). The reduction in oviposition (degree of deterence) was greatest at higher rates (14 ml/Kg) of either oil (Appendix 4.2).

4.3.2.3 S.zeamais: No-choice experiment.
The oils tested had no significant ($P>0.05$) effect on total oviposition (Table 4.6).

4.3.2.4 S.zeamais: Two-way choice experiment.
Groundnut oil, applied at 14 but not at 3.5 ml/Kg significantly ($P<0.001$) reduced total oviposition on treated grains in the two-way choice chamber (Table 4.7). The two-way choice experiments depended on the hypothesis of an equal (50:50) distribution of eggs being laid on any two equal batches of seed within the chamber. This hypothesis was not contradicted in the

Fig. 4.1 Mean ± S.E. eggs laid per day by C.maculatus on treated and untreated cowpea grains (No-choice experiment).

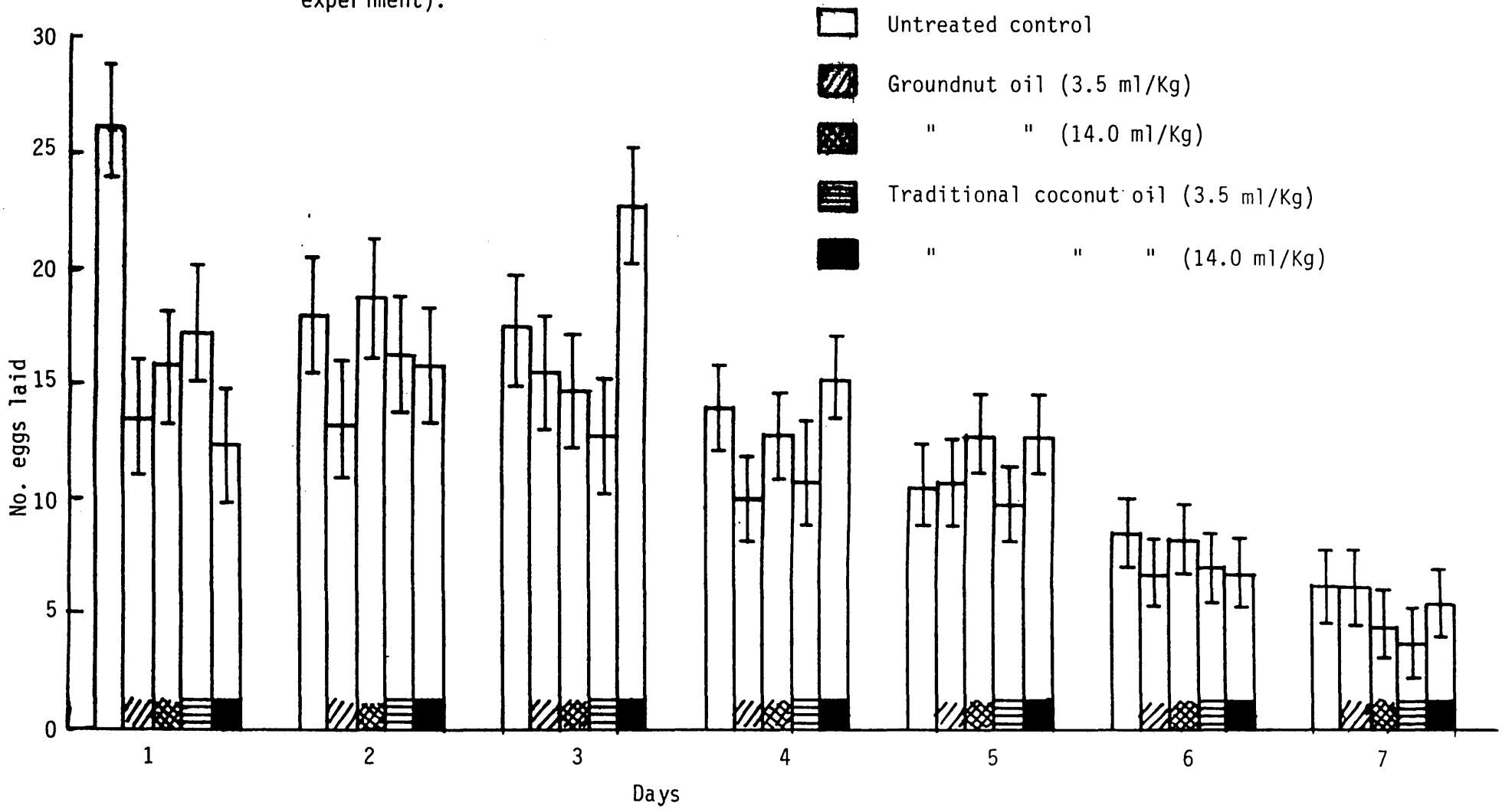


Fig. 4.2 Effects of fixed oils on daily egg laying of C.maculatus in two-way choice oviposition chambers.

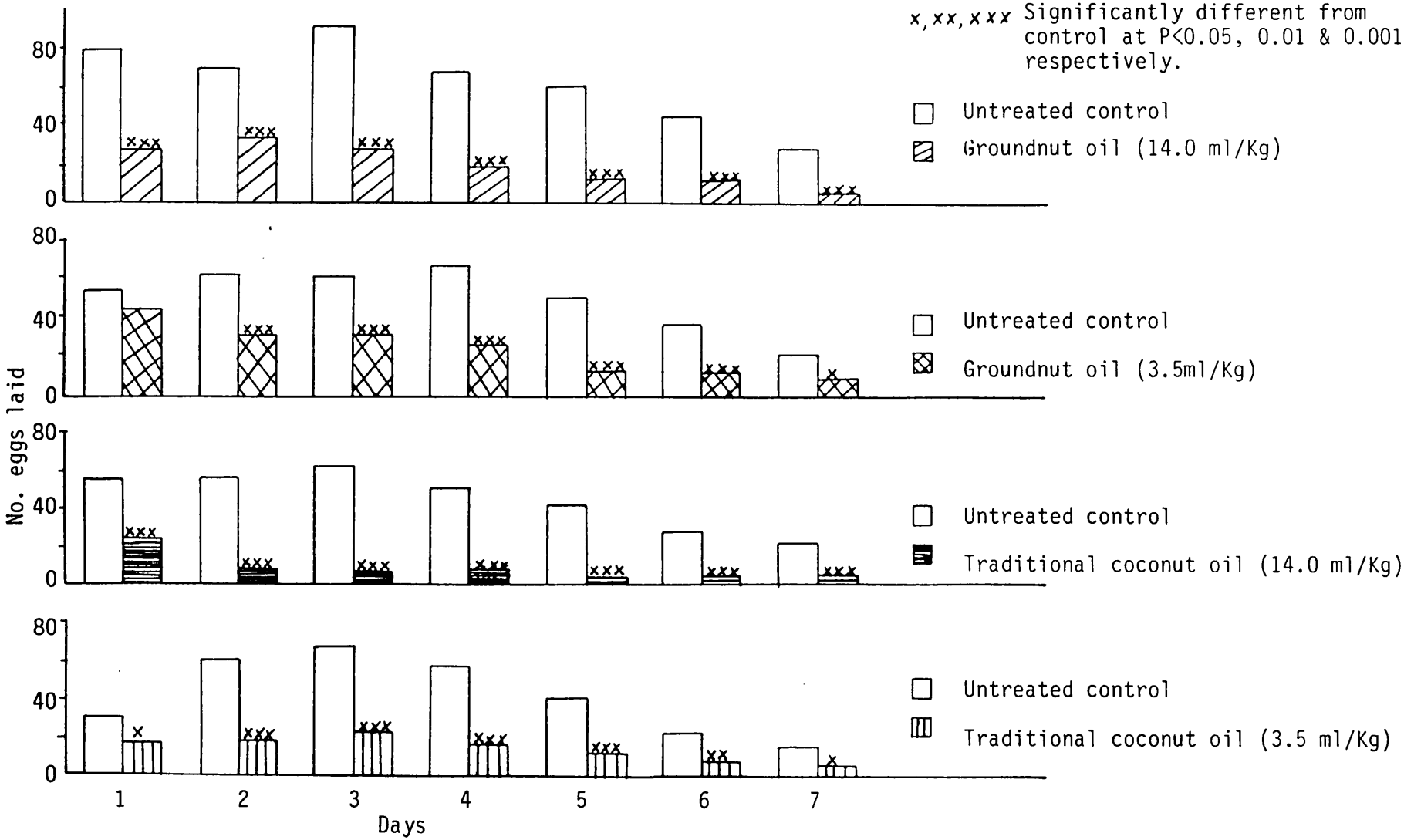


Table 4.6 Effects of fixed oils on oviposition of S.zeamais.

Treatments (ml/Kg)		$\bar{x}$ eggs laid *
Control		82.3
Groundnut oil	(3.5)	88.0
	(14)	98.0
Corn oil	(3.5)	67.3
	(14)	65.0
Traditional coconut oil	(3.5)	80.3
	(14)	80.7
Sesame oil	(3.5)	66.7
	(14)	58.0
Pooled S.E.		8.7

n = 3

* $P > 0.05$.

Table 4.7 Effects of groundnut oil on oviposition of S.zeamais in a two-way choice chamber.

Treatments (ml/Kg)	Total eggs laid	n
Groundnut oil (3.5)	115	5
vs		
Untreated	134	5
Groundnut oil (14)	58***	5
vs		
Untreated	136	5
Controls		
Untreated	39	2
vs		
Untreated	41	2
Groundnut oil (3.5)	21	1
" vs " " (3.5)	20	1
Groundnut oil (14)	8	1
" vs " " (14)	5	1

*** Significantly ($P<0.001$ different from untreated control) (Chi-square).

experiments carried out in this work; the distribution of eggs in control chambers holding pairs of identically treated grains not being significantly (P>0.05) different (Appendix 4.2, Table 4.7).

4.3.3 Ovicidal activity of fixed oils and their major fatty acid components against eggs

4.3.3.1 C.maculatus.

a. Pre-infestation grain treatment. The toxicity levels of groundnut and traditional coconut oils against eggs were similar with overlapping 95% C.L. for the LC_{50} and LC_{95} values (Table 4.8, Appendix 4.3). Joint probit analysis showed that the data did not contradict the model of parallelism.

Of the component fatty acids tested, lauric acid had no significant (P>0.05) effect on eggs (Appendix 4.4). On the basis of LC_{50} values, oleic acid was significantly more toxic than linoleic acid (Toxicity factor = x 7.7) against eggs (Table 4.9, Appendix 4.4). Joint probit analysis showed that the regression lines were parallel.

b. Post-infestation grain treatment. Traditional coconut and groundnut oils appear to be of similar toxicity on the basis of their LC_{50} values (Table 4.10, Appendix 4.5) although the regression lines contradicted the hypothesis of parallelism. In contrast to when fatty acids were applied to grain before infestation (Section 4.3.3.1) acetone-based solutions of lauric, oleic and linoleic acids had similar levels of ovicidal activity based on their LC_{50} values with overlapping 95% C.L. (Table 4.11, Appendix 4.6). Joint probit analysis showed that the regression

Table 4.8 Toxicity of groundnut and traditional coconut oils applied to grains against eggs of C.maculatus.

Treatment	ml/Kg. LC_{50} (95% C.L.)	ml/Kg. LC_{95} (95% C.L.)	Slope ± S.E.+	X^2	D.F.
Groundnut oil	4.54(2.76-6.83)	19.38(10.79-76.6)	2.61 ± 0.79	46.9	2
Traditional coconut oil	5.40(1.94-9.46)	21.01(11.19-95.21)	2.79 ± 0.48	9.71	2

+ Data do not contradict the hypothesis of parallelism.

Table 4.9 Toxicity of fatty acid components of fixed oils applied as grain treatments against eggs of C.maculatus.

Treatment	ml/Kg. LC_{50} (95% C.L.)	ml/Kg. LC_{95} (95% C.L.)	Slope ± S.E.+	X^2	D.F.	T.F.*
Oleic acid	1.64(0.84-2.72)	4.64(2.77-8.56)	3.65 ± 0.66	13.5	2	7.7
Linoleic acid	12.56(8.84-16.22)	31.48(18.29-72.76)	4.12 ± 0.94	9.58	2	1

+ Data do not contradict the hypothesis of parallelism.

* Toxicity factor.

Table 4.10 Toxicity of groundnut and traditional coconut oils applied by dipping against eggs of C.maculatus.

Treatments	(% +) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope ± S.E.++	X^2	D.F.
Groundnut oil	8.77(7.19-10.23)	23.44(19.08-32.41)	3.85 ± 0.54	1.63	1
Traditional coconut oil	9.73(7.86-11.85)	50.65(34.2-100.5)	2.30 ± 0.34	2.39	2

+ Acetone based solutions

++ Data contradict the hypothesis of parallelism.

Table 4.11 Toxicity of component fatty acids of fixed oils applied by dipping against eggs of C.maculatus.

Treatments	(% +) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope ± S.E.++	X^2	D.F.
Lauric acid	3.99(3.12-4.99)	21.68(14.1-47.8)	2.24 ± 0.36	1.83	2
Oleic acid	3.79(3.07-4.55)	14.87(10.98-24.37)	2.77 ± 0.39	0.69	1
Linoleic acid	2.62(1.97-3.43)	19.06(10.91-59.72)	1.91 ± 0.35	2.06	2

+ Acetone based solutions

++ Data do not contradict the hypothesis of parallelism

lines did not contradict the model of parallelism.

4.3.3.2 D.maculatus.

Groundnut oil freshly applied to dried fish surfaces was found to be toxic to eggs (Table 4.12, Appendix 4.7).

4.3.4 Mode of action of fixed oils against C.maculatus eggs

4.3.4.1 Respiratory activity.

The pattern of CO_2 output from eggs laid on groundnut oil-treated grains (n = 299; 3 % hatch) was generally similar to that of eggs on control grains (n = 288; 76 % hatch) for the first 72 h (Fig. 4.3). After this time CO_2 emission from oil-treated eggs continued at a fairly steady but low level in contrast to the sharp increase (over six-fold) observed in the controls for the rest of the monitoring period (Fig. 4.3).

Eggs dipped in groundnut oil (n = 288; <1 % hatch) continued to emit CO_2 at a very low level after treatment compared with the increased emission in the controls (Fig. 4.3).

4.3.4.2 Action on embryonic stages.

It was found that 67 and 48 % of eggs that died on grains treated with groundnut and traditional coconut oils respectively had reached a late embryonic stage compared with only 18 % of the dead eggs in the control treatment (Table 4.13).

4.3.4.3 Effects of solvent on embryonic development.

It was found that the proportion of eggs that had died at a late embryonic stage tended to decrease with increasing amounts of

Fig. 4.3 Pattern of CO_2 emmission from groundnut oil-treated and untreated C.maculatus eggs on cowpea grains.

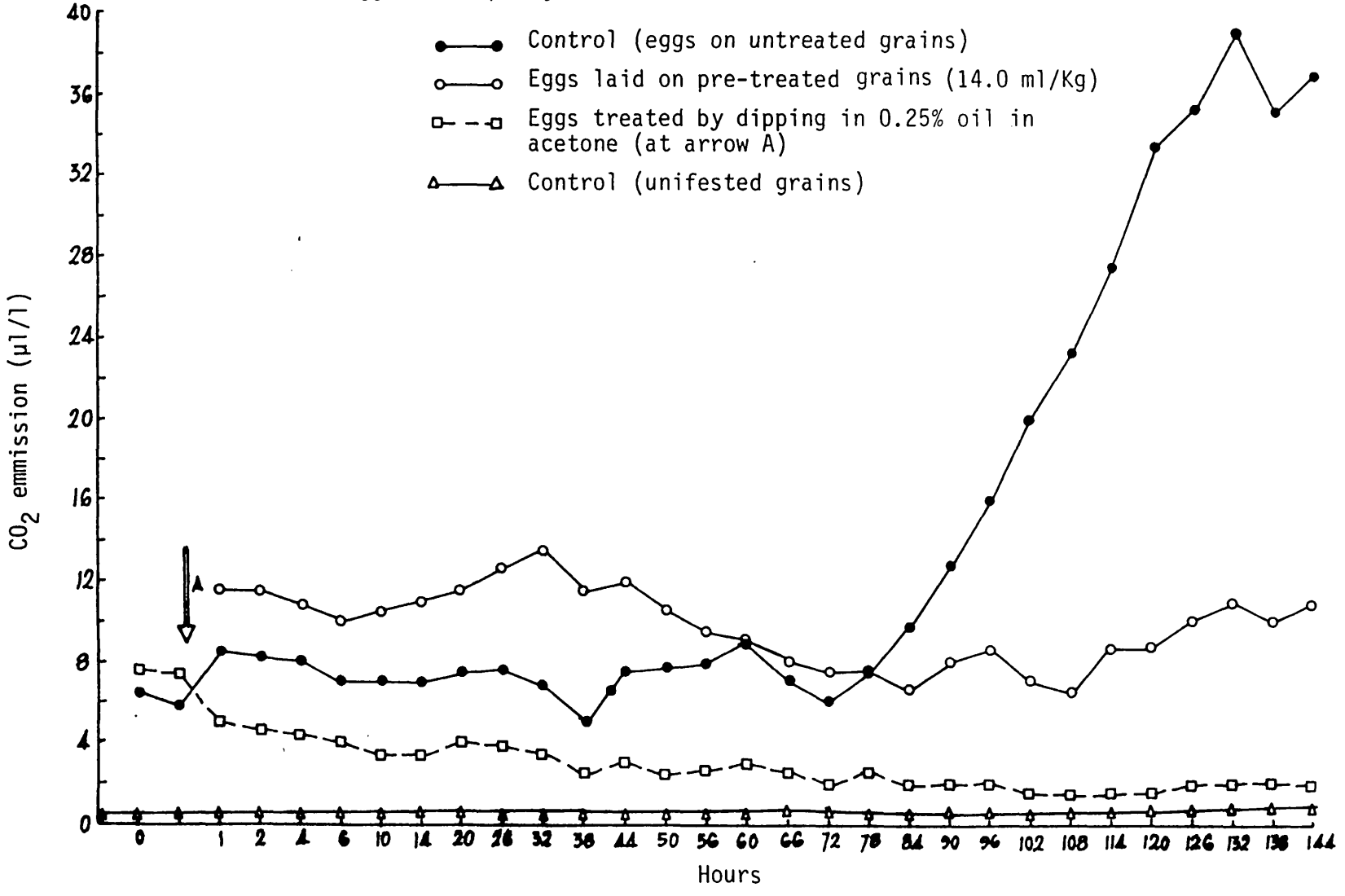


Table 4.12 Toxicity of groundnut oil applied onto dried fish strips against eggs of D.maculatus.

Treatment	ml/Kg. LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope ± S.E.	X^2	D.F.
Groundnut oil	18.29(15.78-20.64)	36.65(29.1-67.6)	5.45 ± 1.33	0.43	1

Table 4.13 Effects of fixed oils on the stage of death of embryos inside eggs of C.maculatus.

Treatments (ml/Kg)	Total Eggs laid	Dead eggs Unhatched	Dead eggs with late embryos (%)+
Control	248	42	8 (18)
Groundnut oil (14)	251	235	157 (67)
Traditional coconut oil (14)	307	269	130 (48)

n = 4

+ % based on total egg mortality.

solvent in the oil solutions tested (Table 4.14).

4.3.4.4 Hatching.

A 2 x 2 contingency Chi-square test showed that there was no significant ($P>0.05$) difference between the proportion of eggs that successfully developed up to the late embryonic stage on oil-treated and untreated Sellotape membranes. However, there was a significant ($P<0.001$) difference between the number of embryos that successfully hatched on the oil-treated surface compared with controls (Table 4.15).

4.3.4.5 Effects on attachment of eggs to grains.

The number of eggs dislodged from treated grains after 4 or 8 min of shaking was significantly ($P<0.001$) greater than in the corresponding control (Table 4.16). In analysing the data, 2 x 2 contingency Chi-square tests were carried out for each replicate separately and then pooled with 2 degrees of freedom (D.F.) after Goodman (1968). Increasing the shaking time appeared to increase the number of eggs dislodged on treated grains to a greater extent than on control grains.

4.3.5 Effects of fixed oils and their major fatty acid components on progeny development

4.3.5.1 *C. maculatus*: Pre and post-oviposition treatment.

Groundnut and traditional coconut oil had no significant ($P>0.05$) effect on the percentage mortality of larvae that emerged from treated eggs (Table 4.17); their action being against the egg stage (Table 4.8, Appendix 4.4).

Table 4.14 Effects of groundnut oil dissolved in acetone on the stage of death of embryos within eggs of C.maculatus.

Treatment (% soln.)	Total Eggs laid	Dead eggs Unhatched	Dead eggs with late embryos (%)+
Control (acetone treated)	74	19	4 (21)
Groundnut oil (100)*	85	85	34 (40)
(50)	94	94	38 (40)
(25)	87	85	30 (35)
(12.5)	66	50	15 (30)
(9.4)	109	87	19 (22)
(6.3)	53	21	4 (19)
(3.2)	62	18	3 (17)

n = 4

+ Based on total egg mortality

* No solvent.

Table 4.15 Effects of groundnut oil on hatching of C.maculatus eggs.

Treatment	Total		
	Eggs laid	late embryos (1st instar)	hatched embryos
Untreated membrane (control)	102	96	96
Groundnut oil treated membrane	143	127	9***

n = 2

*** Significantly different from control at P<0.001 (Chi-square).

Table 4.16 Effects of groundnut oil on the attachment of C.maculatus eggs to cowpea seeds.

Shaking regimes (Time, mins)	Replicates (Experiment)	Untreated control		Groundnut oil(14ml/Kg)	
		Total eggs tested	Eggs attached	Total eggs tested	Eggs attached
2	1	31	28	32	29
	2	40	40	28	28
	3	23	23	23	23
	Totals	94	91	83	80
4	1	31	27	32	22
	2	40	38	28	19
	3	23	23	23	22
	Totals	94	88	83	63***
8	1	31	24	32	11
	2	40	26	28	8
	3	23	22	23	13
	Totals	94	62	83	32***

*** Significantly different from control at $P<0.001$ (Chi-square, 2 D.F.).

Table 4.17 Effect of groundnut and traditional coconut oil on mortality of larvae emerged from treated eggs of C.maculatus.

Treatments (ml/Kg)		$\bar{x}$ Emerged larvae	$\bar{x}$ Dead larvae	% larval mortality[+] P>0.05
Control - untreated		65.0	7.4	9.5 ± 4.7
Groundnut oil	(3.5)	37.6	7.2	16.5 ± 5.2
	(7.0)	28.8	10.2	36.2 ± 7.5
Traditional coconut oil	(3.5)	62.4	12.2	17.9 ± 5.4
	(7.0)	39.0	8.6	20.6 ± 6.3

n = 5

[+] % data transformed angularly.

Table 4.18 Effects of lauric acid applied as grain treatments on C.maculatus adults (parental), oviposition and adult emergence.

Treatments (ml/Kg)		% parent adult mortality[+]	$\bar{x}$ eggs laid ± S.E. P>0.05	$\bar{x}$ adult emergence ± S.E. P>0.05
Control (acetone treated)		44	113.8 ± 15.1	79.0 ± 7.7
Lauric acid[*]	(1.4)	8	126.0 ± 17.0	83.3 ± 7.7
	(1.75)	33	129.0 ± 17.1	84.0 ± 8.8
	(3.5)	0	136.3 ± 15.1	76.0 ± 7.7
	(7.0)	17	100.7 ± 17.1	52.7 ± 8.8
	(10.5)	25	110.7 ± 17.1	61.3 ± 8.8
	(14.0)	13	109.3 ± 15.1	55.8 ± 7.7

[+] n = 16 insects (3 or 4 replicates per treatment

[*] Applied 10% acetone solution.

Pre-oviposition treatments with fatty acids had no apparent effect on parental adult mortality. Oviposition was not significantly ($P>0.05$) affected by the acids nor was there a significant ($P>0.05$) correlation between oviposition and dosage (Tables 4.18 - 4.20).

There was also no significant correlation ($P>0.05$) between adult emergence and dosage in lauric acid treatments, but with oleic and linoleic acids there were significant negative correlations ($P<0.002$ and $P<0.001$ respectively). In addition, analysis of variance showed that the latter two acids significantly ($P<0.001$) reduced adult emergence compared with the control (Tables 4.19 and 4.20); oleic acid being particularly effective.

The above fatty acids had no significant ($P>0.05$) effect on percentage larval mortality, based on number of larvae that successfully hatched from treated eggs (Table 4.21); their action being against the egg stage (Table 4.9; Appendix 4.5).

4.3.5.2 C.maculatus: (10 - 11 day-old larvae).
Groundnut and traditional coconut oils applied to pre-infested grains containing larvae had no apparent effect on larval mortality (Table 4.22). However, there was a tendency towards a greater percentage larval mortality at greater rates (10.5 - 14 ml/Kg) of oil when compared with the control.

In a separate experiment, it was found that when oils were applied at 17.5 ml/Kg larval mortality was much greater than in the control (Table 4.22).

Table 4.19 Effects of oleic acid applied as grain treatments on C.maculatus adults (parental), oviposition and adult emergence.

Treatments (ml/Kg)	% parental adult mortality+	$\bar{x}$ eggs laid ± S.E. P>0.05	$\bar{x}$ adult emergence ± S.E. P<0.001
Control - untreated	31	138.5 ± 14.5	92.0 ± 5.2
Oleic acid (1.4)	25	107.3 ± 16.7	39.7 ± 5.9
(1.75)	33	69.0 ± 16.7	11.0 ± 5.9
(3.5)	19	79.5 ± 14.5	4.0 ± 5.2
(7.0)	33	95.7 ± 16.5	0.0
(10.5)	8	114.0 ± 16.7	0.0
(14.0)	6	125.8 ± 14.5	0.0

n = 16 insects (3 or 4 replicates per treatment).

Table 4.20 Effects of linoleic acid applied as grain treatments on C.maculatus adults (parental), oviposition and adult emergence.

Treatments (ml/Kg)	% parental adult mortality+	$\bar{x}$ eggs laid ± S.E. P>0.05	$\bar{x}$ adult emergence ± S.E. P<0.001
Control - untreated	31	138.5 ± 11.6	92.0 ± 7.2
Linoleic acid (1.4)	33	118.7 ± 13.4	72.0 ± 8.3
(1.75)	17	117.7 ± 13.4	78.0 ± 8.3
(3.5)	6	139.5 ± 11.6	85.0 ± 7.2
(7.0)	25	126.7 ± 13.4	63.0 ± 8.3
(10.5)	17	112.3 ± 13.4	28.7 ± 8.3
(14.0)	6	108.5 ± 11.6	16.8 ± 7.2

n = 16 insects (3 or 4 replicates per treatment).

Table 4.21 Effects of component fatty acids on mortality of larvae emerged from treated eggs of C.maculatus.

Treatments (ml/Kg)		$\bar{x}$ Emerged larvae	$\bar{x}$ Dead larvae	$\bar{x}$ % larval mortality P>0.05 +
Control - untreated		115.5	23.5 ± 6.1	20.6 ± 4.9
Control - acetone treated		108.3	29.3 ± 6.1	25.0 ± 4.6
Lauric acid	(3.5)	124.8	48.8 ± 6.1	39.6 ± 6.0
	(14)	80.5	24.8 ± 6.1	28.1 ± 4.7
Oleic acid	(3.5)	9.0	3.5 ± 6.1	39.6 ± 5.2
	(14)	0	0	0
Linoleic acid	(3.5)	112.8	26.5 ± 6.1	22.0 ± 4.3
	(14)	41.0	14.5 ± 6.1	34.5 ± 5.8

n = 4

+ % data angularly transformed.

Table 4.22 Effects of groundnut and traditional coconut oil applied to preinfested cowpea grains on early stage larvae of C.maculatus.

Treatments (ml/Kg)	$\bar{x} \pm$ S.E.† Larvae/ treatment	Adult emergence	Dead larvae	% larval mortality +
A				
Control	30.8	25.8±1.1	5.0 ± 1.0	15.3 ± 3.2
Groundnut oil(1.75)	30.3	25.8±1.1	4.5 ± 1.0	15.3 ± 3.1
(3.5)	31.0	27.0±1.1	4.0 ± 1.0	12.8 ± 2.9
(7.0)	30.5	25.8±1.1	4.8 ± 1.0	15.3 ± 3.2
(10.5)	30.8	22.8±1.1	8.0 ± 1.0	26.5 ± 3.9
(14.0)	30.5	21.0±1.1	9.5 ± 1.0	31.3 ± 4.0
*(17.5)	30.7	3.0	27.7	90.2
B				
Control	30.8	25.8±1.2	5.0 ± 1.3	15.3 ± 3.8
Traditional coconut oil (1.75)	30.8	26.3±1.2	4.5 ± 1.3	12.8 ± 4.2
(3.5)	30.8	27.3±1.2	3.5 ± 1.3	11.7 ± 3.9
(7.0)	31.0	26.8±1.2	4.3 ± 1.3	12.8 ± 3.5
(10.5)	30.5	25.5±1.2	5.0 ± 1.3	15.3 ± 4.5
(14.0)	30.8	24.3±1.2	6.5 ± 1.3	20.6 ± 4.3
*(17.5)	30.3	10.7	19.7	65.0

† n = 4

+ % data angularly transformed

* Data from a separate experiment (n = 3)

4.3.5.3 C.maculatus: (20 - 21 day-old larvae/pupae).
Increasing rates of groundnut and traditional coconut oils applied to pre-infested grains had little effect on mortality of late larvae/pupae within seeds (Table 4.23).

4.3.5.4 Penetration of oil into cowpea grain.
In assessing the penetration of groundnut oil into treated seeds, it was found that egg mortality on surfaces below the seed coat was comparable with the control (surface of untreated seed) (Table 4.24). Ageing oiled seeds for 10 days before removal of the seed coat and other surface layers did not increase the percentage egg mortality compared with mortality on equivalent surfaces 24 h after oil application (Table 4.24).

4.3.5.5 S.zeamais: (pre-oviposition treatment).
Although oils had no significant ($P>0.05$) effect against oviposition (Table 4.6) they did significantly ($P<0.001$) reduce the percentage adult emergence compared with the control (Table 4.25). There was a highly significant ($P<0.001$) dosage effect whereas the oil main effect was not significant ($P>0.05$).

In acting on progeny development, the test oils did not significantly reduce the percentage adult emergence from pupae but they did significantly reduce the total percentage emergence of larvae from eggs ($P<0.05$) and pupae from larvae ($P<0.001$) (Table 4.26).

4.3.5.6 S.zeamais: (post-infestation treatment, 3 - 14 day-old eggs/larvae.
When groundnut and traditional coconut oils were applied to

Table 4.23 Effects of groundnut and traditional coconut oil applied onto preinfested cowpea grains on late stage larvae/pupae of C.maculatus.

Treatments (ml/Kg)	$\bar{x} \pm$ S.E.† Larvae/ treatment	adult emergence	Dead larvae	% larval mortality +
Control	30.3	27.3	3.5	10.6 ± 3.2
Groundnut oil (3.5)	30.8	27.5	3.0	8.5 ± 2.9
(7.0)	30.5	27.0	3.8	11.7 ± 3.4
(14.0)	30.8	25.5	5.0	16.5 ± 3.2
(17.5)	30.3	21.5	8.8	20.0 ± 5.6
Traditional coconut oil (3.5)	31.0	27.3	3.8	11.7 ± 3.4
(7.0)	30.5	25.3	5.3	16.5 ± 3.9
(14.0)	31.0	26.0	5.0	15.3 ± 3.8
(17.5)	30.5	25.0	5.5	17.9 ± 4.0
		S.E. = 1.0	S.E. = 1.1	

† n = 4

+ % data angularly transformed.

Table 4.24 Mortality of C.maculatus eggs exposed to different layers (1, 2, 3)[+] of cowpea seeds 24 or 240h after groundnut oil application.

Treatment (grain surface)		Total Eggs laid	Total unhatched eggs (egg mortality)	% egg mortality ± S.E.
A				
Control *	(1)	136	5	3.7 ± 0.2
	(2)	89	12	13.5 ± 0.3
	(3)	100	6	6.0 ± 0.2
Groundnut oil (14ml/Kg)	(1)	176	175	99.4 ± 0.1
	(2)	253	30	11.9 ± 0.2
	(3)	214	21	9.8 ± 0.2
(3.5ml/Kg)	(1)	274	68	24.8 ± 0.3
	(2)	162	19	11.7 ± 0.3
	(3)	156	8	5.1 ± 0.2
B				
Groundnut oil(14ml/Kg)	(1)	296	228	77.0 ± 0.2
	(2)	243	9	3.7 ± 0.1
	(3)	195	4	2.1 ± 0.1
(3.5ml/Kg)	(1)	250	95	38.0 ± 0.3
	(2)	213	8	3.8 ± 0.1
	(3)	149	10	6.7 ± 0.2

[+] 1 = intact seeds; 2 = with seed coat peeled off; 3 = with seed coat and top layer of the andosperm removed.

n = 2 or 4 (5 grains per replicate)

A = Bioassay commenced 24h after oil application
B = Bioassay commenced 240h after oil application

Table 4.25 Effect of fixed oils on adult progeny emergence of S.zeamais.

Treatment (ml/Kg)		X		
		Eggs laid	Adult emergence P<0.001	% adult emergence P<0.001+
Control		82.3	65.3	82.1
Groundnut oil	(3.5)	88.0	42.3*	48.3**
	(14)	98.0	6.3***	3.6***
Corn oil	(3.5)	67.3	36.0**	53.5**
	(14)	65.0	1.0***	0.5***
Traditional coconut oil	(3.5)	80.3	47.0*	58.7**
	(14)	80.7	2.0***	2.4***
Sesame oil	(3.5)	66.7	25.3***	31.3***
	(14)	58.0	0.0***	0.0***
		S.E.= 8.7	S.E.=5.3	S.E.=7.3

n = 3

*, **, *** Significantly different from control at P<0.05, P<0.01 and P<0.001 respectively.

+ % data angularly transformed.

Table 4.26 Effect of fixed oils on the survivorship of eggs, larvae and pupae of S.zeamais.

Treatments (ml/Kg)		$\bar{x}$ ± S.E. Larval emergence	% larval+ emergence from eggs P<0.01	Pupal emergence P<0.001	% Pupal+ emergence P<0.001	% adult emergence+
Control		76.3	93.3±3.2	65.7	90.5±6.1	99.9±3.8
Groundnut oil	(3.5)	68.3	78.0±4.3*	43.7*	65.5±9.9**	98.1±7.0
	(14)	63.0	63.8±5.0*	6.7***	5.9±4.9***	67.1±22.0
Corn oil	(3.5)	52.0	78.0±4.3*	37.3**	71.9±9.4*	96.4±9.0
	(14)	40.0	62.1±6.0*	1.0***	1.1±1.8***	100.0±11.0
Traditional coconut oil	(3.5)	68.7	86.0±3.7	50.0	73.5±9.2	94.1±3.0
	(14)	55.3	68.7±4.9*	2.0***	3.6±3.9***	100.0±3.0
Sesame oil	(3.5)	54.7	80.8±4.1*	29.0***	43.0±11.2***	94.9±10.3
	(14)	40.7	70.3±4.8*	0.0***	0.0***	0.0
		S.E.=8.4		S.E.=6.1		

n = 3

+ % data angularly transformed

*, **, *** Significantly different from control at P<0.05, P<0.01 and P<0.001 respectively.

pre-infested wheat grains with early stage larvae/eggs of S.zeamais, the former applied at 7 and 14 ml/Kg significantly ($P<0.01$) reduced adult emergence compared with the control (Table 4.27). Neither oil (at 14 and 17.5 ml/Kg) had a significant ($P>0.05$) effect on adult emergence when applied to late stage larvae/pupae (Table 4.28).

4.3.5.7 D.maculatus: (topical and glass film application). Topically applied groundnut and traditional coconut oil had no apparent toxic effects on D.maculatus larvae over 120 h (Table 4.29). Similarly, exposure to oils on glass surfaces had no effect on 10 - 11 or 22 - 24 day-old larvae over 120 h (Table 4.30).

4.4 Discussion.

In the preceeding chapter it was shown that fixed oils applied to grains and dried fish significantly reduced progeny development of C.maculatus/S.zeamais and D.maculatus respectively. This was in agreement with and an extension of previous work (Mensina and Renwick, 1983; Singh et al., 1978; Schoonhoven, 1978).

However, there is less agreement on the factors involved in the protection of oil-treated seeds. Workers in this field (Singh et al., 1978; Schoonhoven, 1978 Mummigatti and Ragunathan, 1977) have concluded variously that fixed oils may:

1. Increase adult mortality.

Table 4.27 Effect of groundnut and traditional coconut oils applied to preinfested wheat grains on early stage larvae/eggs of S.zeamais.

Treatment (ml/Kg)		$\bar{x}$ adult emergence ± S.E. P<0.01
Control		70.3 ± 4.0
Groundnut oil	(3.5)	67.7 ± 4.0
	(7.0)	48.7 ± 4.0**
	(14.0)	49.0 ± 4.0**
Traditional coconut oil	(3.5)	64.7 ± 4.0
	(7.0)	61.0 ± 4.0
	(14.0)	64.0 ± 4.0

n = 3

** Significantly different from control at P<0.01.

Table 4.28 Effect of groundnut and traditional coconut oil applied to preinfested wheat grains on late stage larvae/pupae of S.zeamais.

Treatment (ml/Kg)	$\bar{x}$ Adult emergence ± S.E. P>0.05
Control	84.0 ± 8.2
Groundnut oil (14.0)	76.0 ± 8.2
(17.5)	61.7 ± 8.2
Traditional coconut oil (14.0)	76.0 ± 8.2
(17.5)	79.0 ± 8.2

n = 3.

Table 4.29 Mortality of 22-24 day-old D.maculatus larvae following topical application of fixed oils.

Treatments (µl/insect)		Mortality (out of 20) 24h	48h	120h
Control		0	0	0
Groundnut oil	(1)	0	0	0
	(2)	0	0	0
Traditional coconut oil	(1)	0	0	0
	(2)	1	2	2

n = 2 (10 insects per replicate).

Table 4.30 Mortality of 22-24 and 10-11 day-old larvae following exposure to groundnut and traditional coconut oil on glass surfaces.

Treatments	Mortality (out of 20) 24h	48h	120h
A			
Control	0	0	0
Groundnut oil+	0	0	1
Traditional coconut oil+	0	0	0
B			
Control	0	0	0
Groundnut oil+	0	0	0
Traditional coconut oil+	0	0	0

n = 2 (10 insects per surface)

+ approximately 0.03ml oil/38.5cm²
A,B, 22-24 and 10-11 day old larvae respectively

2. Reduce oviposition rates.
3. Act as ovicides by physically blocking gaseous exchange of dormant eggs.
4. Interfere with larval development.

4.4.1 Direct toxicity of fixed oils against adults

Neither groundnut nor traditional coconut oil applied to cowpea grains caused adult C.maculatus mortality (Section 4.3.1.1) which is in agreement with Singh et al. (1978). Tikku et al. (1981) also reported that topical application of several vegatable oils, including groundnut and coconut, had no effect on mortality or egg laying capacity of Bruchus chinensis (Bruchidae). In contrast, Hill and Schoonhoven (1981) reported that palm oil killed adults of the bruchid, Zabrotes subfasciatus.

In the present experiments with adult S.zeamais, freshly applied oils caused appreciable mortality only at higher rates (14 - 17.5 ml/Kg). However, this effect disappeared when adults were exposed to treated grains which had been aged for 14 days. Qi and Burkholder (1981) similarly demonstrated that significant mortality occurred only when adults of S.granarius were exposed to grains freshly treated with a high rate (10 ml/Kg) of groundnut oil.

In the above experiments, S.zeamais adults presumably died from oil picked up from the surfaces of freshly treated grains. However, most of the free surface oil is soon adsorbed and bound to the seed coat and endosperm layers. This hypothesis is supported by the observation that the aged-oil treatments

remained effective against adult progeny emergence.

Adult D.maculatus were not affected by topical application of groundnut or traditional coconut oil nor by exposure to surfaces treated with these oils. Thus confirming that such oils have no contact toxicity against active adults (unless trapped in large oil droplets).

It can thus be concluded that at rates of fixed oils which gave effective control of progeny development, adult mortality was not a major factor.

Hewlett (1975) has shown that refined mineral oil can enter the tracheae of Sitophilus spp. causing anoxia and this could also be the mechanism by which the fixed vegetable oils caused some mortality when applied at high rates. In this study, dead insects were certainly found to be heavily coated with oil. Possible differences in cuticle texture and the distribution of bristles could affect the rate of migration of oils over the cuticle of insects. The location and pattern of spiracular openings together with the normal resting position of insects on surfaces (particularly important for small, flattened insects such as S.zeamais) could also play a role in determining oil uptake.

4.4.2 Effects of fixed oils on oviposition

Adult female C.maculatus have a pre-oviposition period of less than a day (Giga and Smith, 1983; present study, Fig. 4.1). In a no-choice experiment the oils were not found to alter the overall pattern of oviposition or the total number of eggs laid on oil

treated seeds; thus comfirming earlier experiments (Chapter 3). The transitory reduction in eggs laid on treated seeds on the first day of the experiment may have been due to fresh oil deposits on the surface of seeds interfering with the movement and/or probing behaviour of ovipositing females. The above results were in general agreement with Periera (1983) who reported that in trials with groundnuts, karite, palm and neem oils only neem significantly deterred oviposition of C.maculatus.

However, the present results contradicted some other no-choice experiments with C.maculatus where interference with oviposition was reported to be one of the mechanisms by which fixed oils protected grains from bruchid infestation (Singh et al., 1978; Schoonhoven, 1978; Mensina and Renwick, 1983). It is possible that differences in the methods of oil application, storage conditions, plus the use of different strains of insects and cowpea varieties may account for the apparent variable effects of fixed oils on oviposition reported in the literature.

The above results raise the question of the detection of oil films on grains by ovipositing females, as this could be an important consideration in designing control strategies involving fixed oils as protectants. The two-way choice experiment revealed that young mated females could detect the presence of oil on grains and they were observed to preferentially oviposit on untreated grains. This effect of oil was apparently dosage dependent; being greater at higher rates of application.

The ability of C.maculatus adults to detect macroscopic differences in seed coat texture has been demonstrated by Nwanze

et al. (1975) who showed that these insects preferentially oviposited on smooth varieties rather than on rough varieties of cowpea.

In the present studies with S.zeamais, it was similarly shown that fixed oils had no significant effect on oviposition in no-choice experiments, but in two-way experiments groundnut oil at 14 ml/Kg significantly deterred oviposition. The effects of oils on Sitophilus species have not been as widely documented as those of bruchids. However, a recent publication by Qi and Burkholder (1981) reported that groundnut oil-treated wheat grains effectively repelled adult S.granarius in two-way choice chambers. Such a repellent effect may also account for the results of the choice experiment in the present work.

Deterrence of oviposition by groundnut oil in choice chambers did not appear to be as strong with S.zeamais as with C.maculatus. However, the difference between species may have been over estimated due to the retention of S.zeamais on one set of grains over the total oviposition period. This may have led to greater egg-laying activity on treated grains.

4.4.3 Ovicidal activity of fixed oils and their major fatty acid components against eggs

With C.maculatus eggs on oil-treated grains or dipped in acetone-based oil solutions, groundnut and traditional coconut oils were found to have similar toxicity levels. This observation supported earlier results (Chapter 3) which showed that fixed oils acted similarly against immature stages of C.maculatus in

reducing progeny development.

Several studies (see Section 4.1) have demonstrated the effectiveness of fixed oils in protecting grains from insect attack but suprisingly very little information exists on the insecticidal activity of their constituent fatty acids and other components. Hill and Schoonhoven (1981) appear to be the only workers to have tested the activity of vegetable oil fractions against insects. They concluded that triglycerides were the active component of fractionated palm oil and cotton seed oil against Z.subfasciatus, and that only some constituent fatty acids were active.

In the present study, the three most common fatty acid components of the fixed oils examined were found to be similarly toxic against C.maculatus eggs in dipping experiments. However, when the acids were applied to cowpea grains while oleic and linoleic acids were active against eggs, the solid lauric acid was not. The last component was applied in acetone which on evaporation left fatty acid crystals which may not have been able to penetrate or spread over the eggs.

In the pre-oviposition grain application experiments oleic acid was significantly more toxic (x 7.7) than linoleic acid against eggs of C.maculatus. This difference when compared with their similar toxicity levels in the dipping experiment is difficult to explain although it is reasonable to assume that differential solubilities of the two compounds in acetone may have affected their ability to penetrate egg membrane or spread over the eggs.

The expected and observed relative potencies of fixed oils and their principal fatty acid constituents (based on the fatty acid composition of groundnut and traditional coconut oils) are given in Table 4.31. In dipping tests, both oleic and linoleic acids in solvent could account for the toxicity level of groundnut oil and lauric acid could explain the toxicity of traditional coconut oil (based in each case on a proportional toxicity basis). However, when applied to grains without solvent only oleic acid could explain the toxicity of groundnut oil and this is likely to be the principal toxic component; the activity of linoleic acid being enhanced by solvent. Lauric acid, the major component of traditional coconut oil was not active against eggs in solid form (as deposits on eggs).

However, while the constituent fatty acids may be responsible for the toxicity of fixed oils against insect eggs, further studies on the action of components in mixtures are necessary in order to fully explain their joint action.

Groundnut oil freshly applied to dried fish strips was shown to be toxic to D.maculatus eggs, thus providing evidence for the mechanism of action of fixed oils in reducing F1 progeny development of this species. However, the LC_{50} value was about 3 fold less than the rate (112 ml/Kg) that gave significant control of D.maculatus progeny in an earlier bioassay which simulated storage conditions (Section 3.2.2). A subsequent experiment in fact showed that eggs were not affected by oil applied at the LC_{50} value when aged for 10 days before bioassay due to absorption of surface oil. This may partly explain the higher rates needed to give control in bioassays where there was a prolonged exposure of

Table 4.31 Comparative toxicities of two fixed oils and their major fatty acid constituents against eggs of C.maculatus.

Component fatty acids	Groundnut oil			Traditional coconut oil		
	% composition+	Relative potency		% composition+	Relative potency	
		Expected 1	Observed 2		Expected 1	Observed 2
A						
Lauric	0	NP	2.2	44	2.3	2.4
Oleic	46	2.2	2.3	6	16.7	2.6
Linoleic	31	3.2	3.3	2	50.0	3.7
B						
Lauric	0	NP	NA	44	2.3	NA
Oleic	46	2.2	2.8	6	16.7	3.3
Linoleic	31	3.2	0.36	2	50.0	0.43

+ % composition after Sheppard et al.(1978)

NP = Not present

NA = Not active

A = applied by dipping - acetone based solutions

B = pre-oviposition grain application

1. Based on % composition

2. Based on LC_{50} ratios.

ovipositing adults to treated fish. Additionally, eggs laid outside treated fish surfaces in bioassay containers were observed to hatch successfully and re-infest treated fish (K. N. Don-Pedro, unpublished data).

The effective use of fixed oils to protect dried fish from infestation by insects will therefore be possible only if regular applications of oil are made in order to maintain free surface oil. In fact, this is what traditional dried fishmongers appear to be doing in Nigeria by their successive sprinkling or brushing of oil onto fish surfaces (K.N. Don-Pedro, unpublished observations).

4.4.4 Mode of action of fixed oils against C.maculatus eggs

Despite their different properties and origin, all the fixed oils tested in the present study were equally effective in reducing survivorship of eggs on treated grains. Based on similar observations, it has been suggested (Hill and Schoonhoven, 1981) that egg mortality was caused by a general physical property of the oil coating rather than a specific chemical action. This idea further stipulates that diffusion of vegetable oils over the egg may block respiration causing death by anoxia (Mensina and Renwick, 1983; Singh et al., 1978).

In this study, measurement of CO_2 emission from eggs of the same age over 6 days has shown that normal respiratory activity was markedly reduced by groundnut oil. This was in agreement with the work of Smith and Pearse (1948) who examined the action of a mineral petroleum oil against eggs of the oriental fruit moth.

They reported an immediate reduction in the respiratory rate which remained below normal levels long after the normal time for hatching. In addition, they demonstrated that it was the prolonged reduction of normal gaseous exchange by oils which was the critical factor causing mortality. On the basis of the data available from the present study there appear to be two possible modes of action which may not be mutually exclusive:

1. Eggs die from lack of sufficient respiratory activity to support development and hatching plus the accumulation of toxic metabolic end-products, both as a result of the oil "barrier effect".

Certainly, oil-treated eggs showed depressed respiration levels after 72 h, the post 72 h period co-inciding with the development of late embryos (1st instar larvae). Also, oil treatments on artificial membranes had no effect on the proportion of eggs that developed to late embryos but significantly reduced the number of embryos that successfully hatched. While a good proportion of eggs that died on oiled cowpea seeds or after dipping in pure groundnut oil developed up to the late embryo stage. These results therefore contradict the alternative theory of an immediate respiratory block which was proposed by some earlier workers (see Singh et al., 1978; Mensina and Renwick, 1983).

2. The direct toxic effect of penetrated oil or oil constituents.

There is no direct experimental evidence to show oils can penetrate eggs. However, the enhancement of toxicity by acetone with some principal fixed oil components plus the increased

proportion of unhatched eggs which had died in early embryonic development when eggs were dipped in oils in acetone provides some indirect evidence.

Most work on oil penetration of eggs appears to have been carried out with mineral oils (O'Kane and Baker, 1935; Smith and Pearse, 1948) with various results and conclusions which may not be of direct relevance to vegetable oils. However, Abro (1985) has shown that the ovicidal activity of the insecticide avermectin B1 against Plutella xylostella was considerably enhanced by the addition of safflower oil although the mechanism by which the enhancement took place was unknown. If oils or some of their constituents do penetrate eggs they may act by a) hardening of the egg shell to prevent hatching, b) interfering with water balance of the embryo, and c) coagulating egg proteins.

Finally, C.maculatus eggs that hatch successfully have to be able to penetrate through the oily seed coat in order to survive. Larson and Fisher (1938) have shown that 1st instar larvae of bruchids cannot enter the seed unless the egg is firmly attached to the seed surface. While Nwanze and Hobber (1976) showed that more 1st instar larvae successfully penetrated the seed coat of smooth rather than on rough seeds which prevented firm attachment of C.maculatus eggs. In the present work, eggs laid on oil-treated seeds were found to be significantly less firmly attached than controls. It is therefore likely that an oily seed coat would inhibit penetration of C.maculatus larvae. These oils probably interfered with attachment of eggs by weakening the

cement with which C.maculatus females attach their eggs to seeds.

4.4.5 Effects of fixed oils and their major fatty acid components on progeny development

Like fixed oils, the fatty acids tested did not influence C.maculatus oviposition (in a no-choice situation), adult mortality or larvae that successfully penetrated treated grain surfaces. Therefore, when applied to uninfested grains the insecticidal action of fixed oils and their major bioactive constituents depends principally on their ovicidal action.

In the present study, the apparent inactivity of fixed oils against C.maculatus larvae may be partly because the oils did not come into direct contact with larvae within the seed endosperm (in post-infestation application). Evidence for this is based on data which shows that the layers of the cowpea seed immediately below oiled seed coats assayed against C.maculatus eggs were found not to be toxic up to 10 days after oil application. On the other hand, the activity of surface oil in causing larval mortality at very high concentrations (17.5 ml/Kg) was due to the oil layer acting as a physical barrier which was found to interfere with the gaseous exchange of penetrated larvae (K.N. Don-Pedro, unpublished data).

The test oils had no contact toxicity against D.maculatus early and late instar larvae. This provided further evidence that apart from the possibility of drowning, causing mortality of active stages of target insects is not one of the mechanisms by which fixed oils bring about a reduction in adult progeny.

In earlier experiments (Section 3) it was shown that fixed oils significantly reduced adult progeny emergence of S.zeamais on wheat but the action of the oils against eggs and larvae which live within the seeds was unknown. Employing X-ray radiographic techniques it was shown that the fixed oils acting against F1 progeny development had no effect on oviposition and the development of adults from pupae but significantly reduced hatching of eggs and further development of early stage larvae. Qi and Burkholder (1981) similarly demonstrated a reduction in progeny of S.granarius but the action of oils against eggs and larval stages of Sitophilus species has not previously been demonstrated.

As ovipositing S.zeamais females drill grains to deposit eggs, some surface oil could penetrate into the grain. Also the waxy plugs with which this species seals its egg holes could be more penetrable by lipophilic oils. Therefore, it is possible that oil may come into contact with eggs and exert lethal action on developing eggs and/or 1st instar larvae by mechanisms similar to those already described for C.maculatus in this section.

MECHANISM OF ACTION OF CITRUS ESSENTIAL OILS AGAINST INSECTS

5.1 Introduction.

In Section 3 it was established that citrus oils reduced F1 progeny development of C.maculatus, S.zeamais and D.maculatus by causing rapid parental mortality. No residual action against immature stages was observed and this together with the known volatility of citrus oils suggested that such oils had a fumigant action. Many workers have reported the contact insecticidal activity of citrus oils (see Section 1) but in no case was vapour toxicity cited as the principal mechanism of action.

In this section experiments were conducted in airtight chambers to establish the vapour toxicity of citrus oils to adults, eggs and other developmental stages of stored product insects and to nymphs and adults of P.americana.

Following gas chromatographic analysis of some test oils (courtesy of Zimmerman Ltd., U.K.) and a review of other citrus oil components (Shaw, 1979), 30 analytical grade components were obtained commercially and tested for fumigant insecticidal activity.

This section also describes joint action studies involving simple mixtures of citrus oil constituents, and mode of action studies using limepeel oil.

5.2 Materials and methods.

5.2.1 Contact toxicity of oils

5.2.1.1 Application to substrate.

For C.maculatus, limepeel and tangerinepeel oils were applied to cowpea (0.86 - 7.0 ml/Kg) following normal procedures (Section 2.2.3.3) about 1 h before bioassay. Twenty treated or untreated grains were confined in Petri dishes (7 cm dia.) with 15, 1 - 2 day-old adults (mixed sexes). Forty-five or 60 insects were tested per treatment. Adult mortality was assessed after 24 h.

With S.zeamais, limepeel and tangerinepeel oils were applied to wheat (1.75 - 10.5 ml/Kg) following standard procedures. Twenty, 1 - 5 day-old adults were confined with 10 g batches of treated and untreated grains. All treatments were replicated 2 or 3 times. Mortality was assessed after 24 h.

With D.maculatus, limepeel oil was applied to 10 g fish strips at several rates between 7 and 56 ml/Kg. Fifteen, 1 - 10 day-old adults (mixed sexes) were confined with treated or untreated fish strips 2 h after application following normal procedures (Section 2.2.3.3). Each treatment was replicated 2 or 4 times. Mortality was assessed after 24 h.

5.2.1.2 Application to D.maculatus.

Limepeel oil was applied topically to 1 - 15 day-old D.maculatus adults (mixed sexes) as described in Section 2.2.3.3 at 0.25, 0.5, 1.0 and 2.0 µl per insect. Fifteen insects were dosed per treatment and each treatment was replicated twice. Two groups of insects were treated as above; one set was left in open honey jars while the other was placed in airtight glass chambers (500

ml) immediately after topical application. Mortality was assessed after 24 h.

Adult D.maculatus were also fumigated in airtight glass chambers (500 ml) with 5 µl of limepeel oil applied to filter paper (3 cm dia.). Another set of adults were treated topically with the oil at 1 µl/insect and similarly confined in glass chambers. Each replicate consisted of 5 insects per chamber and each treatment was replicated six times.

In a follow-up experiment, 15 C.maculatus adults were confined in airtight glass chambers with 5 D.maculatus adults which had been topically treated with limepeel oil (1 µl/insect). Fifteen C.maculatus adults were also fumigated with limepeel oil at 5 µl/500 ml (Section 2.2.3.3). All treatments were replicated twice. Mortality was assessed after 24 h.

5.2.1.3 Toxicity of non-volatile residues.

For C.maculatus, limepeel or orangepeel oil (0.1 ml) was brushed over the surface of a glass Petri dish (7cm dia.) to give a thin film of oil. Treated Petri dishes were left open for 72 h at 30 - 32 °C, after which 15, 0 - 1 day-old adults (mixed sexes) were confined on the treated surface. All treatments were replicated 6 times. Mortality was assessed after 24, 48, 72 and 120 h.

With D.maculatus, batches of fish strips (10 g) were immersed in limepeel oil for 30 seconds. Treated fish strips were then kept at 34 °C in a ventilated oven for 96 h in order to remove volatile fractions. Control fish strips were heated similarly. The fish strips were then allowed to re-equilibriate in the CT room for 7

days prior to bioassay. Four, 1 - 15 day-old adults (2♀, 2♂) were then confined with treated or untreated fish strips for 7 days. All treatments were replicated 3 times. The experiment was evaluated by assessing the number of larvae and the number of emergent adults (Section 2.2.3.3).

5.2.3 Fumigant action of citrus oils

5.2.3.1 Effect of binding of oils on fumigant toxicity.

The following factors were investigated:

1) Treatment surface.
2) Volume of oil.

1) Fifteen adult C.maculatus were fumigated with 6 µl limepeel oil per l (Section 2.2.3.3) with a) oil deposited on the surface of the glass chamber above the fluon mark, or b) with oil deposited on filter paper (3 cm dia.) suspended within the chamber. All treatments were replicated 4 times and mortality was assessed after 24 h.

2) Mixtures (3:2 v/v) of D-limonene or citronellal (two toxic citrus oil components) were formulated with the non-volatile, non-toxic oil n-Decyl alcohol. Fifteen C.maculatus adults (mixed sexes, 1 - 2 day-old) per chamber were fumigated (Section 2.2.3.3) with a) 3 µl of pure D-limonene or citronellal, or b) 5 µl of D-limonene : n-Decyl alcohol, or citronellal : n-Decyl alcohol mixtures. All treatments were replicated 4 times and mortality was assessed after 24h.

5.2.3.2 Adult insects.

For C.maculatus, fifteen, 1 - 2 day-old individually weighed adults (mixed sexes) were fumigated as described previously (Section 2.2.3.3). All citrus oils (Section 2.1.1) were tested initially at 8 µl/l (replicated 4 times) and subsequently between 6 and 10 µl/l (replicated twice). Mortality was assessed after 24 and 48 h of fumigation.

With S.zeamais, fifteen, 1 - 10 day-old individually weighed adults (mixed sexes) per chamber were fumigated for 24 h at rates from 8 to 16 µl/l. Thirty or 45 insects were used per treatment. Mortality was assessed after 24 h.

With D.maculatus, fifteen, 0 - 10 day-old individually weighed adults (mixed sexes) per chamber were fumigated at 20 µl/l (treatment replicated 4 times) and subsequently at rates from 10 - 24 µl/l (treatments replicated twice). Mortality was assessed after 24 and 48 h of fumigation.

5.2.3.3. Immature stages.

a) Eggs. For C.maculatus, two to three day-old eggs on cowpea (Section 2.2.3.3) were fumigated for 24 h with citrus oil at rates from 4 - 16 µl/l. Five egg-infested grains were tested per chamber and all treatments were replicated 2 or 3 times. Egg mortality was assessed 12 days after fumigation (Section 2.2.3.3).

With D.maculatus, eggs (1 - 24 h-old) were fumigated for 24 h with limepeel or orangepeel oils at several rates. Mortality was assessed by counting the number of

eggs that hatched. In a second experiment eggs were fumigated for 48 h.

b) Larvae/pupae. For C.maculatus early stage larvae (11 - 12 day-old), five grains per chamber, pre-infested with larvae were fumigated with limepeel, orangepeel or tangerinepeel oils at rates from 6 - 28 µl/l. After 24 h grains were transferred to ventilated vials. Treatments were replicated 2 or 4 times. Larval mortality was evaluated by assessing adult emergence up to 40 days after oviposition (Section 2.2.3.3).
In a similar experiment, late stage larvae/pupae (20 - 21 day-old) were fumigated at concentrations between 12 and 32 µl/l for 24 h. X-ray radiographs (Section 2.2.3.3) of fumigated and unfumigated cowpea seeds were taken 5 - 6 h before fumigation, at 7 days after fumigation, and on the 38 th day after oviposition. For cowpea seeds, the tube voltage was adjusted to 18 Kv at a maximum tube current of 3 mA with an exposure time of 5 min.

With D.maculatus late stage larvae (20 - 25 day-old), fifteen larvae per chamber were fumigated for 48 h with limepeel or orangepeel oil at rates from 12 to 32 µl/l. Each treatment was replicated twice. Mortality was assessed 48 h after fumigation and this was complimented by counting the number of successful pupations. Larvae were provided with food after fumigation. In a similar experiment, fifteen pupae per chamber were fumigated for 48 h with limepeel or orangepeel oils at rates from 16 -

40 µl/l. Mortality was assessed by counting the number of adults that emerged from treated and untreated pupae.

5.2.3.4 Gas chromatography of citrus oils.

Gas chromatographic (G.C.) analysis was carried out at the Zimmerman Company laboratory, Milton Keynes by courtesy of their analytical chemists. A Perkin Elmer model 8320 was used with a fused silica capillary column (0.22 mm i.d.; 0.33 mm o.d. x 12 m) (SGE code 12QC2) with bonded phase OV101. The carrier gas was helium at 8.3 p.s.i. The capillary inlet and detector temperature were 250°C. Oven temperature was held at 55°C for 5 min then raised to 220°C at 3°C per min.

5.2.3.5 Fumigant toxicity of citrus oil components.

For C.maculatus adults (1 - 2 day-old, mixed sexes), test insects (15 per chamber) were fumigated with each of 30 components selected for initial screening at 10 and 20 µl/l (Section 2.2.3.3). Mortality was assessed after 24 and 48 h fumigation.

In a second experiment, compounds that showed insecticidal activity were re-tested from 2 - 16 µl/l. At least 30 insects were used per treatment. Mortality was assessed after 24 and 48 h fumigation. Solid components were tested at dosages from 4 - 10 g/l (Section 2.2.3.3).

All three stored products species were tested against D-limonene as follows: Test insects (15 adults per chamber) were fumigated for 24 h at several concentrations (C.maculatus 6 - 10 µl/l; S.zeamais 12 - 24 µl/l; D.maculatus 10 - 24 µl/l; see Section 2.2.3.3). Each treatment was replicated twice. Mortality was

assessed after 24 h.

5.2.3.6 Joint fumigation action of citrus oil components.

a) Artificial limepeel oil mixture. This mixture was made using 4 insecticidal components in the same proportions (D-limonene 55.0 %; ɣ-terpinene 10.6 %; Terpinolene 5.3 %; ∝-terpineol 5.8 %) as they occurred in the test batch of limepeel oil (Section 5.2.3.4). N-decyl alcohol, a non-insecticidal component was included as a filler to make up the proportion of the natural oil (23.3 %) occupied by minor components.

Test insects (15, 1 - 2 day-old C.maculatus adults per chamber) were fumigated with the prepared mixture at rates from 6 - 10 µl/l. Each treatment was replicated twice. Mortality was assessed after 24 and 48 h fumigation. For comparative purposes, other batches of insects from the same culture were fumigated simultaneously with natural limepeel oil or mixtures (consisting of each of the toxic components in the artificial limepeel oil mixture with n-decyl alcohol, at equivalent rates to the artificial mixtures).

b) Joint action of binary mixtures of citrus oil components. Mixtures of D-limonene and ∝-terpineol were tested in the following ratios: 4:1; 3:2; 1:1; 2:3 and 1:4. The pure compounds were also tested individually. The same fumigation assay for adult C.maculatus was used as for the artificial limepeel oil mixture. Several rates were tested and mortality was assessed after 24 h.

5.2.3.7. Fumigant toxicity of limepeel oil in the presence of grains and dried fish.

For C.maculatus, fifteen 1 - 2 day-old adults were fumigated per chamber together with measured amounts of uninfested cowpea grains (5, 10, 20, 40, 80 or 160g) using limepeel oil at rates from 6 - 44 µl/l (Section 2.2.3.3). Each treatment was replicated twice. Mortality was assessed after 24 h fumigation.

In a follow-up experiment, insects were fumigated at 10 µl/l in the presence of similar amounts of grain as above. In this case, mortality was assessed after 24, 48, 72 and 96 h fumigation. Since it was impossible to assess adult mortality without removing the grains from the chambers, concurrent experiments were run for each bioassay period.

With D.maculatus, fifteen 1 - 10 day-old adults per chamber with dried fish strips (10 g) were fumigated with limepeel oil at dosages from 150 - 500 µl/l. Each treatment was replicated twice. Mortality was assessed after 24 h fumigation.

5.2.3.8 Mode of action of citrus oil vapour.

a) Speed of fumigant action. For C.maculatus, fifteen, 1 - 2 day-old adults were fumigated with limepeel oil or D-limonene (Section 2.2.3.3) at rates below and slightly above the estimated LC_{95} values. Treatments were replicated twice. Mortality was assessed at regular intervals from 0.5 to 168 h fumigation.

For D.maculatus, a similar experiment was carried out with 1 - 10 day-old adults.

b) Symptoms of poisoning. For C.maculatus, adults (1 - 2 day-old, mixed sexes) were fumigated with limepeel oil at 10 µl/l (0.95 times the LC_{95} value) with either one or 15 insects per chamber (Section 2.2.3.3). Treatments were replicated six times. Insects were observed continuously for the first 3 h and then at 1 h intervals up to 15 h fumigation.

For D.maculatus, a similar experiment was carried out where adults (1 - 10 day-old, mixed sexes) were observed for up to 24 h fumigation.

c) Acetylcholinesterase activity. Ten D.maculatus adults (mixed sexes, 1 - 10 day-old) were fumigated with limepeel oil at 24 µl/l (1.2 times the LC_{95}) for 6 h. Treatments were replicated 4 times. Insects which showed a marked response (partial paralysis) were selected for enzyme assay together with controls.

The heads of ten insects were removed, weighed and then homogenised in 1 ml phosphate buffer (0.1 M), pH 8.0 in an all glass homogeniser immersed in crushed ice. The homogenate was then centrifuged at 6000 rpm for 10 min and the supernatant assayed using the colorimetric method of Ellman et al. (1961). A Beckman DBGT spectrophotometer was used at 412 nM with an ambient temperature of approximately 18°C. The final reaction

mixture contained:

3.0 ml of 0.1 M phosphate buffer, pH 8.0
0.1 ml of 0.01 M dithiobis nitrobenzoic acid (DTNB) prepared in the above phosphate buffer
0.1 ml of enzyme extract (in the test cuvette) or distilled water (in the blank cuvette)
0.1 ml acetylthiocholine iodide in 0.1 M phosphate buffer, pH 7.0.

Absorbence readings were taken at one min intervals for 7 min. Enzyme activity was expressed in absorbence units per min per mg wet weight of insect material since only relative rates of activity were required.

d) Water loss. In a preliminary trial, Periplaneta americana (mixed stages - nymphs and adults) were fumigated with limepeel oil at rates from 16 - 36 µl/l (Section 2.2.3.3). Ten to fifteen insects were used per treatment.

In a second experiment, 5 insects were weighed individually before and after 6 h fumigation at 36 µl/l (1.1 times the LC_{95}).

5.3 Results.

5.3.1 Contact toxicity of oils

5.3.1.1 Application to substrate.

When bioassays commenced 1 h after application to the substrate the citrus oils tested all had similar levels of toxicity to C.maculatus or S.zeamais (Tables 5.1 & 5.2; Appendices 5.1 & 5.2). Limepeel oil appeared to be somewhat less toxic to D.maculatus (Table 5.3; Appendix 5.3) than to C.maculatus or S.zeamais (Tables 5.1 and 5.2) although comparisons must be qualified as joint probit analysis showed the 3 probit lines were not parallel.

5.3.1.2 Direct application.

Topically-applied limepeel oil was shown to be much more toxic to adult D.maculatus when treated insects were enclosed in an airtight chamber (Table 5.4). It was found that when this oil was applied to adult D.maculatus, sufficient oil vapourised to give 100 % mortality of C.maculatus confined in the same fumigation chamber (Table 5.5).

Within an enclosed chamber it was also shown that limepeel oil applied topically (at 1 µl/insect to 5 insects) was significantly ($P<0.001$) less toxic to D.maculatus adults than when the same amount was applied to filter paper within the same size chamber (Table 5.6).

5.3.1.3 Toxicity of non-volatile residues.

The non-volatile residues of limepeel and orangepeel oils on glass surfaces were not found to be toxic to C.maculatus (Table 5.7). Similarly, non-volatile residues of limepeel oil on dried fish strips had no significant ($P>0.05$) effect on progeny development of D.maculatus (Table 5.8).

Table 5.1 Toxicity of citrus oils on cowpea grains against C.maculatus adults.

Treatments	24h (ml/Kg) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.+	X^2	D.F.
Tangerine-peel oil	4.49(2.84-11.95)	9.85(7.49-15.67)	4.16±0.52	6.15	2
Limepeel oil	2.83(2.60-3.07)	5.73(5.04-6.85)	5.37±0.52	3.68	2

+ Data do not contradict the hypothesis of parallelism.

Table 5.2 Toxicity of citrus oils on wheat grains against S.zeamais adults.

Treatments	24h (ml/Kg) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.+	X^2	D.F.
Limepeel oil	4.15(3.90-4.40)	6.39(5.88-7.16)	8.75±0.88	7.60	3
Tangerine-peel oil	5.04(4.65-5.45)	9.14(8.13-10.77)	6.37±0.66	0.77	1

+ Data do not contradict the hypothesis of parallelism.

Table 5.3 Toxicity of limepeel oil on dried fish against adults of D.maculatus.

	24h (ml/Kg)				
Treatment	LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.	X^2	D.F.
Limpeel oil	14.31(12.55-16.0)	38.88(33.16-48.36)	3.79±0.37	1.65	1

Table 5.4 Toxicity of topically-applied limepeel oil to adult D.maculatus in airtight and open chambers.

	24h (µl/insect)				
Treatment	LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.	X^2	D.F.
Limepeel oil (closed)	0.65(0.52-0.88)	1.77(1.19-4.73)	3.79±0.88	0.61	1
Limepeel oil (open)	>2.0[+]				

[+] Size of insect did not permit application of dosages above 2µl/insect.

Table 5.5 Fumigant toxicity of limepeel oil to C.maculatus adults when applied to D.maculatus or on filter paper.

Treatment	30h % mortality (n=30)
Control	0
Topical[+]	100
Vapour ++	100

[+] Applied to 5 D.maculatus adults (1µl/insect) per chamber.

[++] Applied on filter paper - 5µl per chamber.

Table 5.6 Comparative toxicity of limepeel oil when applied topically or as a vapour to D.maculatus adults.

Treatment	24h mortality
Topical[+]	3***
Vapour[++]	16
Control	0

n = 30

*** Significantly different from vapour treatment at $P<0.001$ (2x2 contingency Chi-square test).

[+] 1µl/insect

[++] 5µl/chamber applied on filter paper.

Table 5.7 Toxicity of non-volatile fraction of citrus oils to adult C.maculatus.

	Mortality (n = 90)			
Treatment	24 h	48 h	72 h	120 h
Control	0	1	6	13
Limepeel oil[+]	0	1	3	5
Orange-peel oil[+]	1	2	6	13

n = 6 (15 insects per replicate).

[+] 100µl per glass Petri dish (6.5cm dia.).

Table 5.8 Effects of non-volatile residues of limepeel oil on progeny development of D.maculatus.

	$\bar{x}$		
Treatment	Larvae P>0.05	Emergent adults P>0.05	% adult emergence ± S.E.[+] P>0.05
Control	30.3	14.7	48.3 ± 7.8
Limepeel oil	28.0	13.3	50.0 ± 7.9

n = 3 (2♀ , 2♂ per replicate).

Pooled S.E. of larvae and adults = 4.2 and 2.0 respectively.

[+] % data angularly transformed.

5.3.2 Fumigant action of citrus oils

5.3.2.1 Effect of binding of oils on fumigant toxicity.

a) Treatment surface. The fumigant toxicity of limepeel oil deposited on a glass surface was shown to be significantly ($P<0.001$) greater against C.maculatus adults than when the same amount of oil was deposited on filter paper (Table 5.9).

b) Volume of oil. When greater volumes (5 µl) of 3:2 mixtures of D-limonene/citronellal and the inactive, non-volatile n-decyl alcohol oil were deposited on filter paper significantly ($P<0.001$) greater mortality of C.maculatus adults occurred compared with the corresponding active component alone (3 µl) (Table 5.10).

5.3.2.2 Adult insects.

There was no significant ($P>0.05$) difference between the fumigant toxicity of a single dose of 6 different citrus oils to C.maculatus (Table 5.11) or D.maculatus adults (Table 5.12). These observations were confirmed by subsequent log-dose studies with C.maculatus (Table 5.13; Appendix 5.5), S.zeamais (Table 5.14; Appendix 5.6) and D.maculatus (Table 5.15; Appendix 5.7). Joint probit analysis showed that none of the 3 data sets (within species) contradicted the hypothesis of parallelism.

The relative fumigant toxicity of limepeel oil (as a typical citrus oil) to adults of the 3 test species is given in Appendix 5.16. The toxicity level (LC_{50}) of the oil to C.maculatus was

Table 5.9 Relative fumigant toxicity of limepeel oil applied on glass and filter paper surfaces against C.maculatus adults.

Treatment (6µl/l)	24h Mortality (n=60)[+]
Control	0
Glass	32
Filter paper	5***

[+] 4 replicates (15 insects per replicate).

*** Significantly different from glass at $P<0.001$ (2x2 contingency Chi-square test).

Table 5.10 Fumigant toxicity of pure D-limonene and citronellal and their respective 3:2 mixtures with n-decyl alcohol against C.maculatus adults.

Treatment (µl/l)	24h mortality (n = 60)[++]
D-limonene (6)	4***
D-limonene:n-decyl alcohol - 3:2 (10)[+]	18
Citronellal (6)	24***
Citronellal:n-decyl (10)[+] alcohol - 3:2	60
Control	0

[++] 4 replicates (15 insects per replicate).

[+] 10µl/l with 6µl a.i./l.

*** Significantly different from corresponding 3:2 mixture at P<0.001 (2x2 contingency Chi-square test).

Table 5.11 Fumigant toxicity of citrus peel oils against C.maculatus.

	$\bar{x}$ mortality (n = 15)†	
Treatment oil (8µl/l)	24h P>0.05+	48h P>0.05+
Control	0.0	0.0
Limepeel	6.8	12.5
Tangerinepeel	5.0	9.5
Orangepeel	4.3	8.3
Lemonpeel	4.5	8.8
Grapefruitpeel	3.8	11.0
Mandarinpeel	3.8	8.3

† 4x 15 insects per treatment

+ Pooled S.E. of 24 and 48h mortality data = 5.3 and 1.7 respectively.

Table 5.12 Fumigant toxicity of citrus peel oils against D.maculatus adults.

Treatment oil (20µl/l)	$\bar{x}$ mortality (n = 15)† 24h P>0.05+	48h P>0.05+
Control	0.0	0.0
Limepeel	12.3	12.8
Tangerinepeel	9.8	10.8
Orangepeel	10.5	11.8
Lemonpeel	10.5	11.8
Grapefruitpeel	11.8	12.5
Mandarinpeel	11.3	12.0

† 4 x 15 insects per treatment.

+ Pooled S.E. for 24 and 48h mortality data = 0.89 and 0.73 respectively.

Table 5.13 Fumigant toxicity of citrus oils against C.maculatus adults.

	24h (µl/l)					
Treatment	LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.++	X^2	D.F.	T.F.+
Limepeel oil	7.99(7.66-8.32)	10.49(9.81-11.7)	13.88±1.91	0.68	1	1
Tangerine-peel oil	7.85(7.48-8.23)	10.90(10.04-12.53)	11.53±1.69	0.71	1	1.02
Lemonpeel oil	7.77(7.45-8.08)	10.06(9.46-11.09)	14.67±1.96	0.32	1	1.03
Grapefruit peel oil	8.22(7.91-8.54)	10.44(9.83-11.51)	15.82±2.17	6.34	3	0.97
Orangepeel oil	8.17(7.82-8.54)	10.95(10.15-12.41)	12.96±1.85	1.56	1	0.98
Mandarin-peel oil	8.21(7.85-8.60)	11.16(10.3-12.79)	12.35±1.80	2.68	1	0.98

+ Toxicity factor

++ Data do not contradict the hypothesis of parallelism

$\bar{x}$ adult weight = 4.1 ± 0.2mg (n = 50).

Table 5.14 Fumigant toxicity of citrus oils against S.zeamais adults.

Treatment	24h (µl/l). LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.++	X^2	D.F.	T.F.+
Limepeel oil	11.75(11.25-12.25)	16.16(15.12-17.81)	11.88±1.3	0.94	1	1
Tangerine-peel oil	12.03(11.56-12.51)	16.04(15.09-17.51)	13.18±1.4	0.11	1	0.98
Orangepeel oil	11.44(10.91-11.97)	16.41(15.23-18.32)	10.50±1.2	0.76	1	1.02
Lemonpeel oil	12.56(12.07-13.05)	16.1(15.18-17.65)	15.27±1.9	0.54	2	0.93
Grapefruit peel oil	13.41(12.84-14.06)	18.08(16.68-20.73)	12.69±1.8	4.46	2	0.88
Mandarin-peel oil	12.89(12.19-13.67)	19.3(17.25-23.82)	9.39±1.6	4.26	2	0.91

+ T.F. = Toxicity factor (reference limepeel oil).

++ Data do not contradict the hypothesis of parallelism.

$\bar{x}$ Adult weight = 2.7 ± 0.1mg (n = 49).

Table 5.15 Fumigant toxicity of citrus oils against D.maculatus adults.

	24h (µl/l)					
Treatment	LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E. ++	X^2	D.F.	T.F.+
Limepeel oil	12.72(11.63-13.64)	19.60(17.78-22.97)	8.76±1.30	1.61	1	1
Tangerine-peel oil	11.30(9.51-12.57)	21.50(18.75-27.73)	5.89±1.06	0.13	1	1.23
Orangepeel oil	12.35(10.89-13.50)	22.06(19.43-27.54)	6.53±1.07	0.77	1	1.30
Lemonpeel oil	16.56(15.24-18.46)	28.59(23.72-42.85)	6.94±1.37	0.60	1	1.22
Grapefruit peel oil	15.58(14.19-17.36)	28.82(23.56-45.18)	6.16±1.26	2.25	1	0.91
Mandarin-peel oil	15.18(13.84-16.74)	27.36(22.75-40.62)	6.43±1.26	0.15	1	0.98

+ Toxicity Factor (reference, limepeel oil)..

++ Data do not contradict the hypothesis of parallelism.

$\bar{x}$ Adult weight = 20.4 ± 0.6mg (n = 40).

significantly greater (no overlapping 95 % C.L.) than to either S.zeamais or D.maculatus. Joint probit analysis showed that the 3 log dose-response curves were parallel.

5.3.2.3 Immature stages.

Eggs, larvae and pupae of C.maculatus and D.maculatus were shown to be susceptible to citrus oil vapour (Tables 5.16 - 5.19; Appendices 5.8 - 5.11). Furthermore, examination of X-ray radiographs showed that C.maculatus larvae which died had not developed further than the stage (size and position within seeds unchanged) at which they were 5 - 6 h before fumigation. The second set of X-ray films taken 7 days after fumigation showed some degree of shrinkage due to water loss from affected larvae which was also indicative of rapid mortality.

Relative tolerances based on fumigant LC_{50} values (95 % C.L.) for limepeel oil showed that C.maculatus adults, eggs and early larvae were comparable; being significantly (no overlaps in 95 % C.L.) less than the tolerance of the late larvae/pupae (see Tables 5.13, 5.16 & 5.18). The fumigant toxicity of limepeel oil (LC_{50}) was similar to that of other citrus oils only against adults, eggs and early stage larvae.

D.maculatus adults were significantly less tolerant to limepeel oil than were eggs of this species (no overlaps in 95 % C.L. of 24 h LC_{50} values; Tables 5.15; 5.17); while late stage larvae and pupae were significantly more tolerant than eggs to limepeel oil fumes (48 h LC_{50} - no overlap in 95 % C.L.; see Tables 5.17; 5.19). Limepeel oil fumigant toxicity levels (LC_{50}) were similar to that of other citrus oils only against adult, egg and pupal

Table 5.16 Fumigant toxicity of citrus oils to eggs of C.maculatus

Treatment	24h (µl/l) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.[++]	X^2	D.F.	T.F.[+]
Limepeel oil	7.77(7.11-8.50)	12.94(10.66-15.68)	7.46±1.68	13.57	4	1
Tangerine-peel oil	6.62(5.88-7.31)	14.70(12.07-21.27)	4.75±0.80	3.65	4	1.2
Orangepeel oil	6.60(5.86-7.34)	14.39(11.85-20.35)	4.86±0.77	0.36	3	1.2
Grapefruit peel oil	8.74(7.63-10.04)	16.19(9.53-27.50)	6.16±2.24	0.01	1	0.89
Lemonpeel oil	8.13(7.57-8.73)	11.46(9.35-14.04)	11.04±3.17	0.02	1	0.96
Mandarin-peel oil	7.61(6.84-8.13)	12.02(10.92-14.43)	8.27±1.47	5.27	2	1.02

[+] Toxicity Factor (reference limepeel oil).

[++] Data contradict the hypothesis of parallelism.

Table 5.17 Fumigant toxicity of citrus oils to D.maculatus eggs.

Treatment[+] (h)	µl/l LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	SLope±S.E.*	X^2	D.F.	T.F.[++]
Limepeel oil (24)	21.46(17.94-23.77)	39.43(33.31-59.76)	6.23±1.46	0.11	1	1
Orange-peel oil (24)	21.58(16.63-24.24)	44.78(35.44-101.7)	5.19±1.53	0.22	1	0.97
Limepeel oil (48)	14.65(11.35-16.53)	28.05(22.42-65.67)	5.83±1.81	0.13	1	1
Orange-peel oil (48)	14.87(12.10-16.20)	23.30(19.90-43.70)	8.48±2.73	0.84	1	1.03

[+] Fumigation time before mortality assessment.

[++] Toxicity factor (reference limepeel oil 24, 48).

* 24 and 48h data sets do not contradict the hypothesis of parallelism.

Table 5.18 Fumigant toxicity of citrus oils to early larvae and late stage larvae/pupae of C.maculatus within cowpea grains.

Treatment	24h (µl/l) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.	X^2	D.F.	T.F.+
Early larvae*						
Limepeel oil	9.06(8.14- 9.96)	18.96(15.89-25.81)	5.13±0.78	0.99	2	1
Orange-peel oil	9.47(8.43-10.41)	17.08(15.00-20.92)	6.42±0.89	1.02	1	0.97
Tangerine-peel oil	10.21(8.53-11.38)	17.46(15.60-21.10)	7.06±1.25	0.29	2	0.93
Late larvae/pupae*						
Limepeel oil	17.81(15.10-20.82)	53.18(38.90-101.5)	3.46±0.65	1.35	2	1
Orange-peel oil	12.37(10.53-13.82)	27.83(22.58-43.47)	4.67±0.93	0.50	1	1.50
Tangerine-peel oil	11.72(9.17-13.64)	38.33(28.75-71.25)	3.20±0.63	1.06	2	1.48

+ Toxicity Factor (reference limepeel oil).

* Data sets do not contradict the hypothesis of parallelism.

Table 5.19 Fumigant toxicity of citrus oils to late larvae and pupae of D.maculatus.

Treatment	48h (µl/l) LC_{50} (95% C.L.)	LC_{95} (95% C.L.)	Slope±S.E.	x^2	D.F.	T.F.+
Late larvae*						
Limepeel oil	23.14(20.19-27.04)	64.40(46.71-129.6)	3.70±0.73	0.78	2	1
Orange-peel oil	16.53(13.72-18.99)	42.52(32.34-80.28)	4.01±0.86	1.50	2	1.40
Pupae**						
Limepeel oil	23.95(20.74-26.52)	47.89(41.31-61.88)	5.47±0.87	0.26	2	1
Orange-peel oil	19.56(15.72-22.38)	51.15(40.47-84.50)	3.94±0.79	1.46	2	1.14

+ Toxicity Factor (reference limepeel oil).

*, ** Data sets do not contradict the hypothesis of parallelism.

stages.

5.3.2.4 Gas chromatography of citrus oils.
It was found that D-limonene was the major component of all test oils (Appendix 5.12).

5.3.2.5 Fumigant toxicity of citrus oil components.
Out of 30 components tested, 22 (including D-limonene) were shown to have strong insecticidal activity against C.maculatus adults (Tables 5.20 & 5.21; Appendices 5.13 & 5.14). The 24 h LC_{50} values of the liquid components ranged from 4.05 (α-terpineol) to 13.13 (n-tridecane) µl/l; while solids ranged from 4 - 10 mg/l.

D-limonene was shown to have a similar toxicity to S.zeamais and D.maculatus but was significantly more toxic to C.maculatus (Table 5.22, Appendix 5.15).

5.3.2.6 Joint fumigant action of citrus oil components.
a) Accounting for the fumigant toxicity of a citrus oil).
Substitution of the LC_{50} values for the components of limepeel oil against C.maculatus (Table 5.20) and their % occurrence into equation (1) (Section 2.2.4) gives an estimate of the LC_{50} of the untested proportion of the oil as 9.84 µl/l (assuming additive action). This untested fraction comprised approximately 25 % of the natural limepeel oil.

b) Artificial limepeel oil mixture. The variance of the LC_{50} value and that of the slope of the probit line for the artificial limepeel oil mixture were not significantly ($P>0.05$) different from those of the natural oil (Table 5.23; Appendix 5.17). None

Table 5.20 Fumigant toxicity of liquid citrus oil components against C.maculatus adults.

Treatment	24h Mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope ± S.E.	X^2	D.F.	% in oil	Expected toxicity* ratio
Limepeel oil ++	7.77(7.11-8.50)	12.94(10.66-15.68)	7.46 ± 1.88	13.57	4	-	1
Liquid components**							
D-limonene	8.28(7.93-8.67)	11.15(10.30-12.73)	12.74 ± 1.85	3.07	1	54+	1.9 (0.9)
Citronellal	5.52(5.00-5.90)	8.86(8.00-10.38)	7.99 ± 1.14	7.39	3	.09+	>1100 (1.4)
n -decyl alcohol	-	-	-	-	-	<0.1	>1000
n-tri-decane	13.13(12.45-14.43)	18.29(16.00-25.20)	11.43 ± 2.48	0.30	1	<0.1	>1000 (0.6)
Nonane	-	-	-	-	-	-	-
n-undecane	10.44(9.74-10.85)	13.11(12.19-16.33)	16.68 ± 4.5	0.09	1	0.03	>3300 (0.7)
α-pinene	10.21(9.88-10.57)	12.73(12.00-13.98)	17.16 ± 2.4	0.19	1	1.7+	59 (0.8)
α-terp-inene	8.03(7.75-8.33)	9.97(9.30-10.66)	17.61 ± 2.7	6.16	2	1.3+	77 (1.0)
β -myrcene	9.61(9.33-9.89)	11.44(10.97-12.20)	21.80 ± 2.9	1.13	3	1.3+	77 (0.8)

Table 5.20 continued.....

Treatment	24h Mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope ± S.E.	X^2	D.F.	% in oil	Expected toxicity ratio *
Citral	4.18(3.70-4.60)	7.78(6.76-9.85)	6.11 ± 0.9	1.21	1	<0.1	>1000 (1.7)
6-methyl-5-hepten-2-one	4.52(4.21-4.82)	6.86(6.23-7.97)	9.07 ± 1.2	1.42	1	<0.1	>1000 (1.7)
γ-terpinene	5.65(5.13-6.03)	8.69(7.91-10.3)	8.79 ± 1.5	0.46	1	10.4+	10 (1.4)
Geraniol	13.06(12.48-13.75)	18.03(16.47-21.09)	11.73 ± 1.7	0.98	1	0.1+	1000 (0.6)
n-decyl-aldehyde	-	-	-	-	-	<0.1	>1000
Linalool	5.16(4.91-5.41)	6.67(6.28-7.37)	14.81 ± 2.1	0.89	3	0.2+	500 (1.5)
Toluene	-	-	-	-	-	-	-
Acetone	-	-	-	-	-	-	-
Undecenal	12.21(11.32-14.73)	18.88(15.33-36.95)	8.69 ± 2.38	0.13	1	<0.1	>1000 (0.6)
β-pinene	8.37(8.03-8.70)	10.67(9.99-12.02)	15.56 ± 2.53	3.76	2	2.3+	44 (0.9)
Terpinolene	6.36(5.89-6.86)	10.46(9.12-11.98)	7.63 ± 1.12	10.24	3	5.2+	19 (1.2)
Myrcene	9.40(9.04-9.73)	11.91(11.28-13.03)	15.97 ± 2.26	0.69	1	1.3	77 (0.8)
Nonanol	-	-	-	-	-	0.5+	200

Table 5.20 continued.....

Treatment	24h Mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope ± S.E.	X^2	D.F.	% in oil	Expected toxicity ratio *
9-decenal	7.29(6.82-7.79)	10.52(9.11-12.13)	10.38 ± 2.1	6.20	1	0.03	>3300 (1.1)
Methyl-anthranilate	5.21(4.88-5.52)	7.64(7.01-8.77)	9.92 ± 1.4	2.23	3	0.03+	>3300 (1.5)
∝-terpineol	4.05(3.56-4.48)	8.06(6.60-12.62)	5.50 ± 1.14	5.67	2	5.7+	18 (1.9)

+ Proportions in oil obtained from G.C. analysis (Appendix 5.12). Others (not determined) obtained from literature after Shaw (1979).

* Expected toxicity ratio = $\frac{100\%}{\%\text{ of component in oil}}$ (toxicity ratio if component alone were toxic in oil).

++ Limepeel oil - typical citrus oil - as reference.

( ) observed toxicity factor (limepeel oil as reference).

** Data contradict the hypothesis of parallelism.

- Where - equals not active.

Table 5.21 Fumigant toxicity of solid citrus oil components against C.maculatus adults.

	24h Mortality ($\bar{n}$ = 30)				
Treatment	4(mg/l)	6(mg/l)	10(mg/l)	0.0	% in oil
Camphene	0	3	4	0	0.56[+]
Fenchyl alcohol	23	30	30	0	0.81
Caprylic acid	NA			0	<0.1
Thymol	NA			0	<0.1
(-)Borneol	0	9	4	0	0.6

[+] Proportion in oil obtained by G.C. analysis (Appendix 5.12). Others not determined obtained from literature after Shaw (1979).

NA Not active against C.maculatus.

Table 5.22 Relative fumigant toxicity of D-limonene against test insects.

Treatments	24h Mortality (95% C.L.) LC_{50}(µl/l)	LC_{95}(µl/l)	Slope±S.E.[++]	X^2	D.F.	T.F.[+]
C.maculatus						
D-limonene	8.28(7.93-8.67)	11.15(10.30-12.73)	12.74±1.85	3.07	1	1
S.zeamais						
D-limonene	16.22(15.35-16.94)	22.04(20.54-24.87)	12.35±1.90	2.73	2	0.51
D.maculatus						
D-limonene	15.35(14.32-16.46)	23.16(20.58-28.87)	9.21±1.56	3.42	2	0.54

[+] Toxicity Factor (reference D-limonene - C.maculatus).

[++] Data do not contradict the hypothesis of parallelism.

Table 5.23 Fumigant toxicity of artificial limepeel oil mixture and natural limepeel oil to C.maculatus adults.

Treatment	24h Mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope±S.E.++	X^2	D.F.	T.F.+
Artificial limepeel oil mixture*	8.65(8.35-8.97)	10.64(10.04-11.77)	18.31±2.8	0.70	2	1
Limepeel oil	8.09(7.78-8.40)	10.27(9.68-11.30)	15.81±2.1	0.23	1	1.07

+ Toxicity Factor (artificial oil as reference).

++ Data do not contradict the hypothesis of parallelism.

* Artificial limepeel oil = mixture of D-limonene (55%), ɣ-terpinene (10.6%), Terpinolene (5.3%), ∝-terpineol (5.8%) and n-decyl alcohol (23.3%).

of the bioactive components included in the artificial oil by themselves or when mixed with the inactive n-decyl alcohol, produced a response (mortality) on C.maculatus adults proportional to the expected response on a % composition basis (Table 5.24; Appendix 5.13).

c) Joint action of binary mixtures of citrus oil components. Isoboles for mixtures with greater amounts of D-limonene fitted the sub-additive model, while isoboles for mixtures with equal amounts of the compounds and greater amounts of α-terpineol did not contradict the model of synergistic action (Fig. 5.1; Appendices 5.18; 5.20).

Bioassay data for D-limonene and α-terpineol, both alone and as a 1:1 (v/v) mixture, was re-analysed using Hewlett and Plackett's model for completely similar action of two components with non-parallel log dose - probit regression lines (Model A, Section 2.2.4). The fit of the experimental data to the maximum likelihood regression lines for D-limonene and α-terpineol estimated for the data by the method was subjected to a Chi-squared test of goodness of fit and gave a significant result ($P<0.001$; Appendix 5.19).

5.3.2.7 Fumigant toxicity of limepeel oil in the presence of grains and dried fish.

With grains or dried fish, the fumigant toxicity of limepeel oil against C.maculatus or D.maculatus adults respectively was reduced (Table 5.25, 5.26; Appendices 5.21, 5.22, 5.23). For example, the presence of 10 g of fish reduced the fumigant toxicity (LD_{50}) of limepeel oil to D.maculatus 19 fold, while 160

Table 5.24 Relative fumigant toxicity of artificial limepeel oil mixture and its active constituents to C.maculatus adults.

Treatment	24h Dosage % mortality response (n=30)		
	8µl/l	9µl/l	10µl/l
Artificial limepeel oil+	20	27	97
D-limonene (55%): n-decyl alcohol (45%)	0	0	20
ɣ-terpinene (11%): n-decyl alcohol (89%)	0	0	0
Terpinolene (5%):n-decyl alcohol (95%)	0	0	0
α-terpineol (6%): n-decyl alcohol (94%)	0	0	0

+ Artificial limepeel oil = Mixture of D-limonene (55%), ɣ-terpinene (11%), Terpinolene (5%), α-terpineol (6%) and n-decyl alcohol (24%).

Fig. 5.1 Isobolograms depicting the amount of each toxicant in the LC_{50} values of limonene - α-terpineol mixtures.

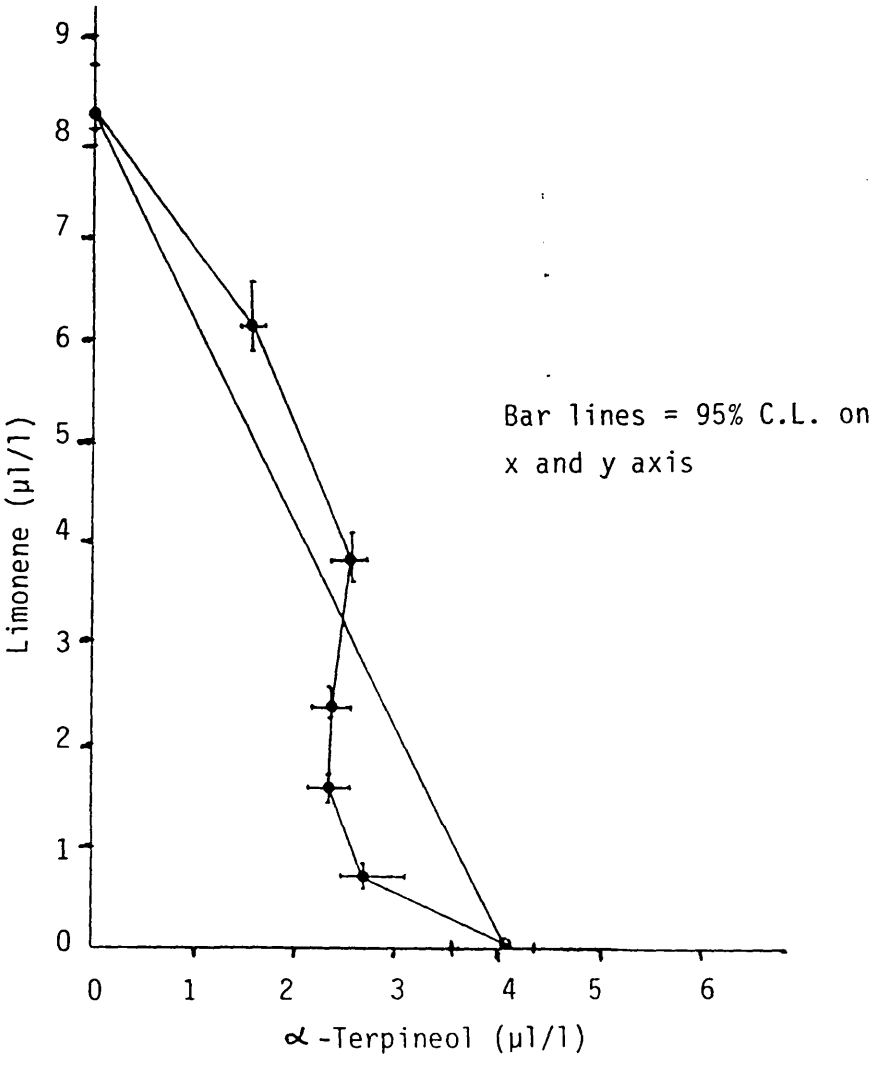


Table 5.25 Fumigant toxicity of limepeel oil against C.maculatus in the presence of varying amounts of cowpea grain.

Treatment (amount of grains (g))		24h Mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope ± S.E.++	X^2	D.F.	T.F.+
Limepeel oil	(0)	7.99(7.66-8.32)	10.49(9.81-11.70)	13.88 ± 1.9	0.68	1	4.0
Limepeel oil	(5)	9.46(9.19-9.73)	11.13(10.70-11.84)	23.36 ± 5.6	5.61	3	3.4
Limepeel oil	(10)	9.95(9.62-10.29)	12.21(11.47-12.98)	18.54 ± 2.9	4.54	3	3.2
Limepeel oil	(20)	10.57(10.20-10.92)	12.70(12.13-13.58)	20.64 ± 2.6	2.48	3	3.0
Limepeel oil	(40)	11.55(10.94-12.08)	16.23(14.75-19.95)	11.13 ± 2.2	0.85	3	2.8
Limepeel oil	(80)	19.71(18.55-20.91)	29.91(26.78-36.95)	9.09 ± 1.5	2.25	1	1.6
Limepeel oil	(160)	31.95(30.20-33.80)	48.98(44.19-58.00)	8.87 ± 1.2	2.10	2	1.0

+ Toxicity Factor (limepeel oil - 160g grains).

++ Data contradict the hypothesis of parallelism.

Table 5.26 Fumigant toxicity of limepeel oil against D.maculatus adults in the presence of dried fish.

Treatment (amount of fish, g)	24h Mortality (95% C.L.)					
	LC_{50} (µl/l)	LC_{95} (µl/l)	Slope ± S.E.	X^2	D.F.	T.F.+
Limepeel oil (0)	12.72(11.63-13.69)	19.60(17.78-22.97)	8.76 ± 1.3	1.61	1	19
Limepeel oil (10)	247.00(214.3-274.5)	565.5(472.9-774.6)	4.57 ± 0.7	4.20	2	1

+ Toxicity Factor (limepeel - 10g fish as reference).

g of cowpea reduced the toxicity of limepeel oil to C.maculatus by approximately 4 fold. In the experiment with C.maculatus, the fumigant toxicity did not decrease proportionately with increasing amounts of grain (Appendix 5.21).

In another series of experiments, it was shown that mortality tended to increase with time when C.maculatus adults were exposed to limepeel oil vapour in the presence of varying amounts of cowpea (Fig. 5.2; Appendix 5.24).

5.3.2.8 Mode of action studies with citrus oil vapour.
a) Speed of fumigant action. The fumigant LT_{50}, and more especially the LT_{95} values for limepeel oil and D-limonene decreased with increasing concentrations applied to both C.maculatus and D.maculatus adults (Tables 5.27 & 5.28; Appendices 5.25 & 5.26). The speed of fumigant action of limepeel oil and D-limonene against each species was similar at comparable rates of application.

b) Symptoms of poisoning. Continuous observation of C.maculatus adults fumigated at 10 µl/l (0.95 times LC_{95} dose) with limepeel oil showed that symptoms of poisoning commenced within the first two min of treatment in the following sequence:

1. Agitation - fast antennal movements and running around substratum.
2. Uncordinated movements - fast leg movements but no progression in apparent attempts to climb the wall of the fumigation chamber.
3. Rolling over/knock down - insects occasionally remaining on

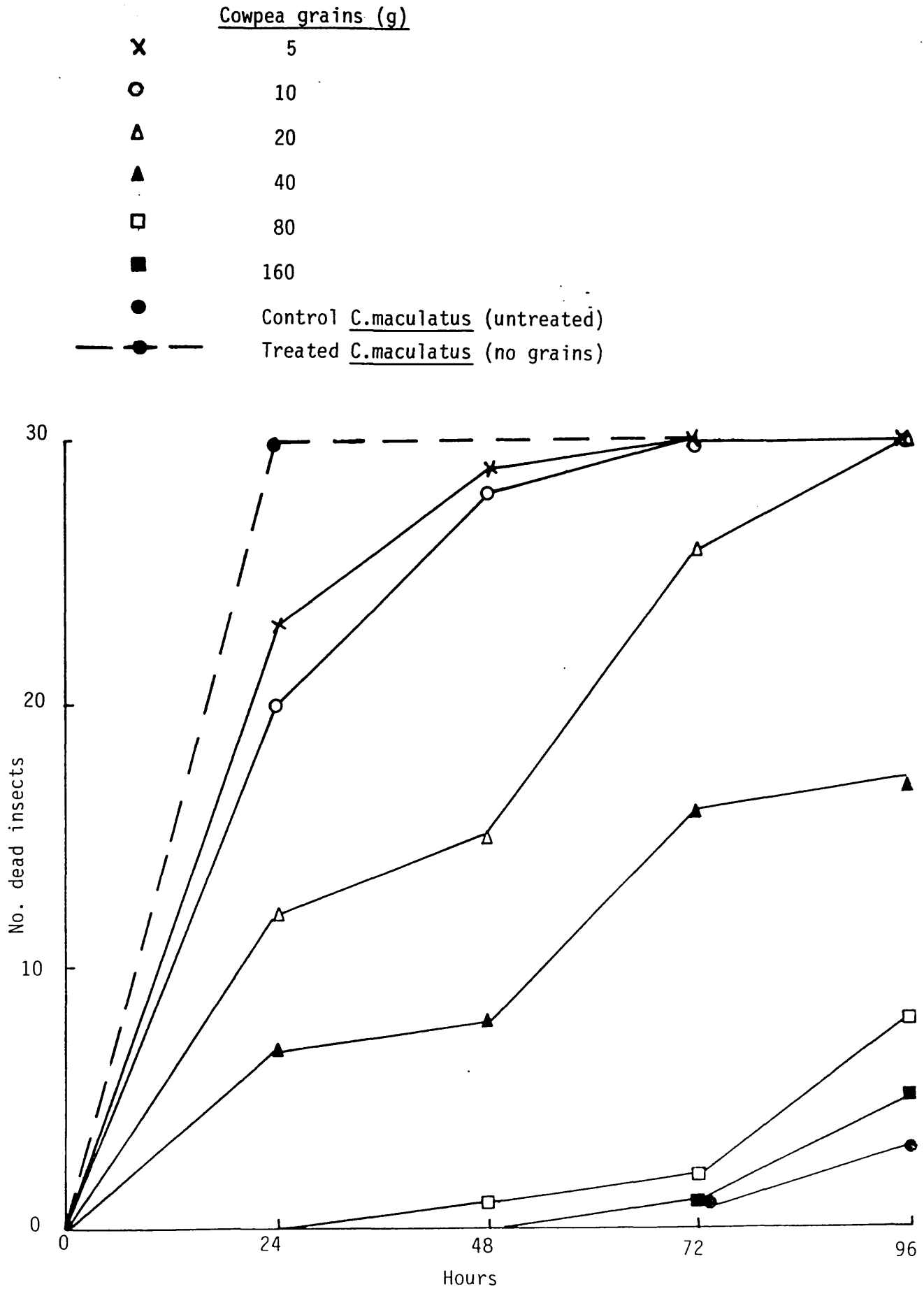


Fig. 5.2 Mortaility of adult C.maculatus exposed to limepeel oil (10 µl/l) vapour in the presence of varying amounts of cowpea grains.

Table 5.27 Speed of fumigant action of limepeel oil and D-limonene against C.maculatus adults.

Treatment (µl/l)+	LT_{50} (h) (95% C.L.)	LT_{95} (h) (95% C.L.)	Slope±S.E.	X^2	D.F.
Limepeel oil (6)	164.4(132.0-352.0)	501.2(275.0-540.5)	3.40±1.22	0.74	2
(8)	40.73(36.40-46.00)	88.59(71.80-127.0)	4.88±0.72	1.60	2
(9)	26.13(20.96-34.99)	165.7(91.90-574.0)	2.05±0.39	0.49	3
(10)	7.33(5.95- 9.02)	38.58(25.10-84.50)	2.28±0.36	5.07	3
(14)	3.75(3.17- 4.39)	12.32(9.39-18.90)	3.18±0.40	1.69	2
D-limonene (6)	162.5(145.6-206.1)	266.9(209.0-672.0)	4.38±2.30	1.54	2
(8)	41.42(37.50-46.10)	76.92(64.50-105.0)	6.12±0.95	0.35	1
(9)	35.18(29.90-41.80)	110.7(81.10-192.7)	3.31±0.51	0.65	1
(10)	4.61(2.12- 8.42)	28.28(12.68-150.0)	2.08±0.50	10.58	4
(14)	2.95(2.49- 3.43)	9.79(7.42-15.62)	3.16±0.45	0.62	2

+ Limepeel oil LC_{50} and LC_{95} values = 7.99 and 10.49µl/l respectively.

D-limonene LC_{50} and LC_{95} values = 8.28 and 11.15µl/l respectively.

Table 5.28 Speed of fumigant toxicity of limepeel oil and D-limonene against D.maculatus adults.

Treatment+ (µl/l)	LT_{50} (h) (95% C.L.)	LT_{95} (h) (95% C.L.)	Slope±S.E.	X^2	D.F.
Limepeel oil					
(10)	106.4(70.70-262.2)	1536(468.0-5942)	1.42±0.39	0.35	1
(16)	11.00(8.0-14.60)	112.6(63.20-321.0)	1.63±0.26	1.01	3
(22)	6.75(4.71- 8.90)	57.66(31.60-219.0)	1.77±0.36	0.82	2
(24)	14.06(12.30-16.60)	38.20(28.30-66.30)	3.79±0.60	0.96	2
(28)	12.04(10.78-13.50)	26.80(21.90-37.50)	4.73±0.45	0.56	1
D-limonene					
(12)	66.10(54.50-87.90)	283.9(170.0-885.0)	2.60±0.52	1.93	2
(16)	14.75(9.36-22.60)	330.4(125.0-3380)	1.22±0.27	3.17	3
(22)	13.12(9.29-20.49)	187.9(75.00-1708)	1.42±0.30	0.47	3
(24)	13.60(12.10-15.68)	33.57(25.70-55.80)	4.19±0.69	0.65	3
(28)	11.00(9.70-12.43)	28.16(22.20-42.80)	4.03±0.62	0.37	2

+ Limepeel oil LC_{50} and LC_{95} values = 12.72 and 19.60µl/l respectively.
D-limonene LC_{50} and LC_{95} values = 15.35 and 23.16µl/l respectively.

their backs for a few seconds.

4. Rolling over and staggering movements - increased regularity; accompanied all the time by fast antennal and leg movements.
5. Rolling over now accompanied by occasional opening and closing of wing - insects spending more time in knock down position.
6. Almost permanently in knock down position - on their backs with progressively slower leg, antennal and wing movements.
7. Paralysis - insects permanently on their back with only occasional slow movements of legs (followed by death).

All affected insects went through most of the above sequence of symptoms but the length of time each stage lasted was more variable. The time interval between the first symptoms and the death of the insect varied from less than 1 h to over 12 h at the approximate LC_{50} level. Similar symptoms were observed in insects treated singly or in groups of 15. Control insects were generally stationary; moving their antennae only occasionally during the period of observation.

D.maculatus adults when fumigated at 24 µl/l (1.2 times LC_{95} dose) showed similar symptoms to C.maculatus. Young larvae (<10 days old) were instantly paralysed by topical application of 1 µl of limepeel or orangepeel oil, while older larvae (16 - 20 day-old) were thrown into several looping movements before dying.

c) Acetylcholinesterase (AChE) activity. No effect on AChE activity was observed with D.maculatus adults (Table 5.29).

d) Fumigant toxicity against cockroaches and water loss. Tentative 24 h LC_{50} and LC_{95} values for fumigant toxicity of limepeel oil against cockroaches are given in Table 5.30 (Appendix 5.27).

The live weight loss per unit body weight of cockroaches fumigated at 32 µl/l (approximately LC_{95} dose) for 6 h was significantly ($P<0.001$) greater than in the control (Table 5.31).

5.4 Discussion

There is relatively little information on the insecticidal activity of citrus oils in the literature, although a number of reports exist on the insecticidal activity of essential oils from other plant sources. In only a few cases has the possible fumigant action of essential oils been discussed (see Section 1).

In Section 3, data was presented to show that the highly volatile citrus oils significantly reduced progeny development of stored product insect pests by causing rapid parental mortality. Subsequent experiments have shown that the major mechanism of action of these oils is by fumigant rather than contact toxicity.

In the present and many other laboratory studies on fumigants, small experimental chambers have been used. Page and Lubatti

Table 5.29 Acetylcholinesterase activity in limepeel oil vapour-treated and untreated adults of D.maculatus.

Treatment (µl/l)	AChE activity* Experiment 1	Experiment 2
Control (untreated)	0.0146	0.0161
Limepeel oil (24)+	0.0156	0.0164

+ 1.2 x LC_{95} dose.

* Absorbance units/min./mg head.

Table 5.30 Fumigant toxicity of limepeel oil to cockroaches.

Treatment	24h mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope±S.E.	X^2	D.F.
Limepeel oil	18.71(16.15-21.67)	32.22(24.20-42.80)	6.99±2.1	0.96	2

Table 5.31 The effect of limepeel oil vapour on body weight of cockroaches.

Treatment (µl/l)	$\bar{x}$ weight	
	Loss (mg)	Loss per unit body weight (mg/mg) P<0.001
Control	11.20 ± 2.6	0.011 ± 0.003
Limepeel oil (32)+	61.40 ± 2.6	0.062 ± 0.003

n = 5

+ Approximately equal to the LC_{95} dose.

(1933) pointed out that the major problem with small chamber experiments was one of accurate dosage determination under standard conditions of temperature and humidity. However, these authors concluded that in spite of their limitations small chambers are an economic way of screening novel fumigants and are capable of showing both the relative toxicity of fumigants to insects and the effects of changes in concentration with time for any particular combination of insect and fumigant.

In simple fumigation chambers, liquid dosing is generally on a piece of filter paper in order to increase the rate of evaporation (Busvine, 1971; Ellis and Morrison, 1967), although it is possible that adsorption of liquids on cellulose fibres may affect the dose in such cases (Busvine, 1971). In the present work, experiments in which varying volumes of oils were applied on filter paper, or where their toxicity on glass and filter paper was compared, suggested that a proportion of the oils did in fact remain bound. The effect of this would be to distort the dosage scale and this may have reduced the response of the test insects. This factor may also have contributed to the significant heterogeneity in a few of the data sets that were subjected to probit - log-dose regression analysis.

Therefore, although the data obtained indicates the relative differences in toxicity between oils and oil components and this data is reproducible (see Appendix 5.28), the reported toxicity levels are not absolute measurements but rather are underestimates. In this work, it was decided to use the term LC_{50} rather than LD_{50} as although not entirely appropriate the former gives a closer approximation to the method of application.

Due to technical limitations, it was not possible to use the more accurate method of volumetric dosing of insects in airtight chambers by conducting known concentrations of oils already in the gaseous state.

Contact toxicity of oils

Although contact toxicity was demonstrated in the present work (as shown by earlier workers, notably Su et al., 1972b, 1976), it was also found that oil-treated grains which caused 100 % mortality 1 h after application lost all activity within 24 h (see Section 3). These results together confirm the non-residual nature of the insecticidal properties of citrus oils.

Topical toxicity trials also illustrated the relative unimportance of the contact toxicity of citrus oils, as appreciable mortality at application rates of up to 2 µl/insect was obtained only when treated insects were confined in airtight chambers. Thus, the toxic volatile components of the oil apparently evaporated into the surrounding air space faster than they penetrated the insect cuticle. This assumption is further supported by the mortality of untreated C.maculatus adults confined in airtight chambers with topically-treated D.maculatus adults.

Therefore, earlier workers (Su et al., 1972a, 1976; Sheppard, 1984; Bestman et al., 1984) who measured the topical toxicity of citrus and non-citrus essential oils in open systems may have measured only a fraction of their true toxicity. Contact toxicity

for these types of plant oils is thus rather incidental and only effective before the toxic volatiles evaporate fully from the surface of application. Certainly, no evidence was obtained for toxic, non-volatile limepeel oil residues on either glass surfaces or dried fish muscle.

Fumigant toxicity of oils

The present study showed that all the citrus oils tested were of the same order of toxicity for each insect species. This contradicts results from earlier experiments in Section 3. However, in the latter work bioassays were conducted in Petri dishes and commenced several hours after oil application when differential rates of evaporation of toxic fractions of the oils may have occurred. The similar toxicity of different citrus oils would appear to be related to the similar range of mostly terpenoid compounds (Shaw, 1979) that constitute the oils.

Joint probit analysis for the six citrus oils tested for vapour toxicity against each insect species showed that each of the 3 data sets did not contradict the hypothesis of parallelism. Some authors (eg. Bliss, 1939, 1954; Horsfall, 1945) have used similar findings to support the likelihood of such compounds exerting similar biological action. However, more recently it has been shown that parallelism is not a criterion for similarity of action (Hewlett and Plackett, 1959, 1979) which can be extended to mean similar biological action, viz. penetration, site of action, metabolism etc. Therefore, parallelism only means that the log tolerance distribution for the two or more compounds has the same variance. This does not contradict similar action

where it exists but offers no proof of similarity and only more biological and biochemical investigations can substantiate or reject this hypothesis.

In this study, it was shown in several experiments with limepeel oil and adult C.maculatus that 48 h fumigation mortality data was more closely related to end-point mortality than 24 h data. In spite of this, most log-dose probit responses were computed using 24 h mortality data. This was because 48 h fumigation periods would have meant using dosages of oils that would have been too small to measure accurately. However, the 24 h data were reasonably reproducible (see Appendix 5.28). In addition, when all the citrus oils under investigation in this work were tested for vapour toxicity against adult C.maculatus and D.maculatus at single dosages, the respective responses of the test insects were not significantly different after 24 or 48 h fumigation.

Fumigant toxicity (LC_{50}) of limepeel oil against D.maculatus was similar to that against S.zeamais adults and only slightly less (1.6 times) than that against C.maculatus. On the basis of adult weight, D.maculatus was the largest species examined with a mean weight that was 5 times and 7.7 times greater than that of C.maculatus and S.zeamais respectively. It therefore appears that size had little effect on susceptibility to the vapour action of citrus oils.

Yao and Yang (1984), working with a non-citrus essential oil (pheasant pepper seed oil) demonstrated vapour toxicity to a bruchid beetle (Bruchis rufimanus) at levels (LC_{50} = 59.6 ml/m^3) of a similar level to those presented in the present work.

However, Taylor and Vickery (1974) observed that 3 ml of orangepeel oil placed on cotton wool killed C.maculatus adults confined within 830 ml cages. It is unlikely that this high rate of application gave a true representation of the vapour toxicity of citrus peel oils as the LC_{50} values obtained for fumes of such oils (tested against adults of C.maculatus) in the present study were about 500 times lower. Also the results of Sheppard (1984), who demonstrated the vapour toxicity of materials from scarified fresh orange peels against house flies were not comparable with the fumigant toxicity of extracted citrus peel oils demonstrated in this work.

Fumigant toxicity against immature stages

The data presented here are the first known to the author that show a significant fumigant ovicidal and larvicidal action of citrus oils against insect stages within grains. Eggs and larvae that died from citrus oil fumigation appeared dried up and brittle with some eggs (of D.maculatus, in particular) appearing dark brownish and shrivelled. These observations were suggestive of water loss. The demonstrated activity of citrus oils against immature stages is indirectly supported by a recent review by Greany et al. (1983) where it was stated that citrus plants were resistant to tephritid fruit fly infestation because citrus oils were toxic to eggs and larvae.

The fumigant activity of oils against C.maculatus larvae within cowpea grains was particularly interesting. Earlier work applying such oils directly onto infested cowpeas was shown to kill larvae but required much larger dosages (eg. LC_{50} = 1.32 ml of limepeel

oil per Kg cowpea, K.N. Don-Pedro, unpublished).

In preliminary trials, S.zeamais larvae within wheat grains were also shown to be susceptible to toxic vapour of citrus oils at concentations less than 25 µl/l (1.5 times LC_{95} dose to adults).

X-ray studies revealed that larval stages within grains died soon after they were exposed to lethal concentrations of oil vapour; suggesting a similar response to that of adults. This rapid action is advantageous since fumigation with citrus oils could be seen as having the potential of arresting feeding activity of larvae immediatley after exposure, thereby providing the best chance of reducing damage in storage.

The active D.maculatus larvae and immobile pupae also appeared to have died rapidly, with no appreciable development after treatment. On the other hand, larvae that received only sub-lethal doses appeared to have developed into adults that looked physically normal.

The relative susceptibility of different larval instars of test species was not investigated in detail but the general trend was that eggs were more susceptible than early stage larvae, and late stage larvae/pupae.were the most tolerant to citrus oil vapour. However, it is noteworthy that these changes in susceptibility based on LC_{50} values were not dramatic for either C.maculatus or D.maculatus. Larval instars and/or weight are known to significantly affect tolerance to many synthetic insecticides (Aikins, 1981). For example, Aikins and Wright (1985) observed a direct relationship between toxic doses of DDT or malathion and

body weight of progressive larval instars of Mamestra brassicae.

Fumigant toxicity of citrus oil components

Out of thirty components tested, 22 including the principal constituent of all citrus oils, D-limonene, were shown to have strong fumigant insecticidal activity against C.maculatus adults.

Joint probit analysis showed that the data for the bioactive liquid components contradicted the model of parallelism. However, for practical purposes α-terpineol was taken to be the most toxic liquid component with a toxicity level (LC_{50}) to adult C.maculatus 2 times greater than D-limonene and 3.2 times greater than that of the least toxic component, n-tridecane (with no overlap in 95 % C.L. (Table 5.20).

Taylor and Vickery (1974) have reported limonene to be the most toxic citrus peel oil component against C.maculatus and houseflies when applied by a dry film (filter paper) method in Petri dishes. On the other hand, Styer and Greany (1983) showed that when applied by dipping, citral was considerably more toxic than limonene to the Caribbean fruit fly, Anastrepha suspensa.

All bioactive components (liquids and solids) were found to be volatile at 28 °C. This supports the idea that the insecticidal activity of citrus oils depended on their volatile components. However, their bioactivity did not depend merely on the physical property of volatility at NTP. In that case, all volatile compounds would have been expected to be toxic which was not the case.

Accounting for the toxicity of a citrus oil

Fumigant toxicity levels (LC_{50} values or % mortality) indicated that none of the individual components tested in this study had a high enough toxicity level against C.maculatus to account for the observed toxicity of limepeel oil on a proportional toxicity basis (see Tables 5.20 and 5.21).

The LC_{50} of the various components of citrus oils varied between 4.05 and 13.13 µl/l; thereby ranging from below to above the LC_{50} value (7.99 µl/l) of limepeel oil (a typical citrus oil). The LC_{50} of the untested proportion, estimated under the supposition of additive action of the components, was slightly higher than that of the oil and there was thus no reason to suppose that this fraction of the oil contained compounds significantly more toxic than those tested. Additive action would seem a reasonable assumption for components with a similar chemical constitution. However, the situation with citrus oils remains unclear, for while the components of natural citrus peel oils are of varied chemical composition most are terpenoids.

Furthermore, bioassays using artificial and natural limepeel oils gave similar levels of toxicity to C.maculatus adults. Whereas, none of the constituents of the artificial oil alone or mixed with the non-toxic component, n-decyl alcohol, could account for the observed toxicity of the mixture on a proportional basis. Additive or synergistic action are possible explanations for the joint action of the components in the artificial limepeel oil mixture. Harbourne (1977) observed that frequently, mixtures of

several closely related structures of the same class of compounds (alkaloid, terpenoid or flavonoid) are produced by plants for their defence and suggested that synergism occurs. One substance enhancing the effectiveness of a second as an insect feeding deterrent. The fact that the artificial limepeel oil with only four bioactive constituents was as toxic as the natural oil is of practical interest. Further work could produce combinations of two or more components that significantly synergise their individual action against insects.

Joint action of binary mixtures of citrus oil components

Bioassay data for D-limonene and α-terpineol by themselves and as a 1:1 (v/v) binary mixture was not consistent with the hypothesis of completely similar action (additive action). On the other hand, the fit of the data did not show any systematic trend away from the regression lines as would have been expected if synergistic or antagonistic effects had been operating. Similarly, when the expanded set of toxicological data involving four additional mixtures were re-analysed as isoboles, the data fit was consistent with sub-additive and synergistic models only in parts. Considering experimental error, the actual isobole may not be significantly different from that of additive action. Although D-limonene and α-terpineol are both monoterpenes, and as such may be assumed to act similarly, it must be emphasised that the modes of action of these compounds in the vapour phase to C.maculatus are unknown. Further work is therefore necessary to substantiate or reject the tentative hypothesis of additive action.

Fumigant toxicity of citrus oil in the presence of grains and dried fish

Volatile components of oils may be taken up by solids during fumigation so that there may be a substantial fall in the concentration in the vapour phase. This phenomenon is described as sorption. Sorption is due to several causes including 1) adsorption; 2) absorption or solution, and 3) chemical reaction (Busvine, 1971).

Bioassay results in this work showed that sorption of citrus oil vapour occurred in the presence of grains or strips of dried fish and that this tended to reduce their toxicity. However, this effect did not increase proportionally with increasing amounts of grain.

Dried fish muscle appeared to be considerably more sorptive of citrus oil vapour than were grains. The greater surface area of fish strips in relation to grains could be a contributory factor as well as quantitative differences between animal and plant tissues, viz penetration/absorption of these vapours.

There was also a tendency for adult mortality to increase with time in the presence of cowpea grains. This was an indication that toxic vapours continued to diffuse through inter-grain air spaces and within the grains themselves. These observations would suggest that citrus oil components do not enter into chemical reaction with, or remain bound to, the grain material. The tentative conclusion from this is that sorption of citrus fumes by cowpeas is governed primarily by absorption rather than

adsorption (condensation of molecules on surface) or chemical reaction. However, only more biological studies backed up by quantitative observations on the rate of uptake of test vapours by grains, fish muscle and other materials would lead to a more definite characterisation of their sorption and subsequant bio-availability in storage systems.

Possible mode of action of oil fumes

On the basis of speed of action, the citrus oils tested are fast-acting insecticides. Interestingly, the LT_{50} and LT_{95} values obtained by Yao and Yang (1984) for pheasant pepper oil against B.rufimanus were much greater (87 and 335 h respectively) than the values obtained in this work (see Tables 5.27 & 5.28). Although the results are not exactly comparable, this may reflect a greater speed of action for citrus oils than other essential oils.

The fast fumigant action of citrus oils suggests that they may affect the insect nervous system and insects fumigated in this study showed symptoms reminiscent of nervous intoxication. In particular, knock down and paralysis occurred rapidly when insects were exposed to lethal concentrations of citrus oil fumes. Rapid action including knock down is considered to depend upon a combination of potent neurotoxicity and rapid distribution through the insect tissues to the site of action (Ford et al., 1981; Szydlo et al., 1985).

There are no known previous investigations into the mode of action of citrus oils against insects. In preliminary studies,

P.americana adults at various stages of paralysis due to fumigation with limepeel oil vapour (LC_{95} level) were dissected for electrophysiological examination of ventral nerve cord activity. It was found that spontaneous and evoked nervous activity consisting of trains of action potentials were similar to that in untreated insects (K.N. Don-Pedro, unpublished data). Thus, no evidence of a neuroactive action was demonstrated in this preliminary trial. The present studies have shown that fumigant toxicity of limepeel oil was also not related to inhibition of AChE activity in D.maculatus.

However, the above results do not preclude citrus oils from acting on the insect nervous system, for example at neuromuscular junctions and further work is necessary.

Neurobiological effects are not always correlated with the overall toxicity of a compound (Leake et al., 1979) and death may occur as a result of the metabolic disruption and tissue damage caused by the uncoordinated release of neurohormones from a poisoned nervous system. For example, a variety of synthetic organic insecticides are known to disrupt insect hormone levels (Maddrell and Reynolds, 1972).

Gerolt (1976) found that the neurotoxic pyrethroid, organochlorine, organophosphate and carbamate insecticides all caused accelerated water loss from Musca domestica. Water loss may occur through the mouth, anus, spiracles or general cuticle. Casida and Madrell (1977) showed that enhanced water loss through the anal opening of allethrin poisoned Rhodnius prolixus was brought about by the excess release of a diuretic hormone by the

neuroendocrine system. However, water loss alone is unlikely to be the sole cause of death since P.americana and M.domestica exposed to dry air until water loss reduced their body weight by up to 15% (the range of loss recorded from pyrethroid poisoned insects described by Hose, 1984) did not die (Ingram, 1955).

In the present study, a significant reduction in body weight caused by water loss from citrus oil vapour-poisoned cockroaches was observed. Lohar (1982) also found that pupae of Tenebrio molitor lost significant amounts of their body weight due to water loss following DDT and malathion poisoning.

The outlet and mechanism of water loss from P.americana in this study was not investigated but P.americana and other insects poisoned by conventional synthetic insecticides are known to lose water by evaporation through the spiracles and body surface (Samaranayaka, 1977; Chittoraj and Sharma, 1964).

The survival of insects depends on the water proofing properties of the cuticle and on the control of excretory and respiratory mechanisms (Maddrell and Casida, 1971) and this critical water balance in insects may be disrupted by insecticides as discussed (see also Maddrell and Reynolds, 1972). However, the rapid insecticidal action of citrus oil vapour in this study suggests that water loss is unlikely to be the primary cause of death but rather the result of several metabolic disruptions. Such metabolic changes could involve the release of a diuretic hormone.

GENERAL DISCUSSION AND CONCLUSIONS

6.1 Fixed and essential oils as alternative insecticides.

In the present study, fixed and essential oils were shown to be potentially useful stored product insecticides; although when applied directly to grain/dried fish, the toxicity of the oils was lower than that of various commercial insecticides (Table 6.1).

Fixed oils were found to act almost exclusively against insect eggs. Oviposition behaviour and other mode of action studies with C.maculatus and S.zeamais (Section 4) showed that complete coverage of fixed oils over the surfaces of grains was necessary to maximise their effectiveness. The use of a solvent was found to enhance activity and/or spread. An automated method of oil application was adapted to give acceptable oil spread on grains in this work (Section 2) but manual mixing (grains) or brushing (dried fish) could be aa effective in spreading oil on substrate if carried out thoroughly over a prolonged period.

A severe limitation with fixed oils was that the rates required to disinfest grains (>14 ml/Kg) were impracticable and it was concluded that such oils were most suited for prophylactic rather than curative use although it has been shown that surviving C.maculatus adults produced significantly less progeny as their

Table 6.1 Estimated toxicity of some conventional insecticides and plant oils when applied to stored produce.

Compounds	LC_{50}† except where indicated*	Substrate	Species	Stage treated	Ref.
Permethrin	4.86	Wheat	T.castaneum	Adult	1
Deltamethrin	20.2	Wheat	T.castaneum	Adult	1
Cypermethrin	25.11	Wheat	S.granarius	Adult	2
Pyrethrins	7.59-8.38	Cowpea	Z.subfaciatus	Adult	3
	6.65	Cowpea	Callosobruchus chinensis	Adult	3
Malathion	8.0*	Maize	S.zeamais	Adult	4
Pirimiphos methyl	5 - 10*	Maize	S.zeamais	Adult	4
Tetrachlorvinphos	20*	Maize	S.zeamais	Adult	4
Limepeel oil	2.4	Cowpea	C.maculatus	Adult	5
	3.57	Wheat	S.zeamais	Adult	5
	2.3	Dried fish	D.maculatus	Adult	5
Groundnut oil	3.5	Cowpea	C.maculatus	Eggs	5
	14.44	Dried fish	D.maculatus	Eggs	5
	11.06*	Cowpea	C.maculatus	Adult	5
	11.06*	Wheat	S.zeamais	Adult	5
	44.24 - 88.5*	Dried fish	D.maculatus	Adult	5

* >95% control of F1 progeny development.

1 = Singh (1981); 2 = Hose (1984); 3 = Weaving (1970); 4 = Weaving (1975); 5 = Present study.

† LC_{50} for commercial pesticides in mg/Kg; for oils in g/Kg.

eggs failed to hatch on the oil-treated (7 - 14 ml/Kg) grains (K.N. Don-Pedro, unpublished data). Fixed oils have in fact been reported to protect cowpea grains against C.maculatus for up to 180 days (Singh et al., 1978).

The fumigant toxicity of citruspeel oils has been unequivocally established for the first time in this work. Vapour toxicity was shown to extend to all life stages including those living within grains (Section 5). The two previous reports that referred to the vapour toxicity of citrus oils gave only limited evidence which failed to bring out their practical value (Section 5.4).

In contrast to fixed oils, the effectiveness of citrus peel oils was found to depend mainly on their strong fumigant action (Section 5). Thus, essential oils should ideally be used within airtight containers although due to their rapid action against insects, semi-airtight containers (which may be easier to obtain in villages) could be effectively used.

Although the relative susceptibility of different insect life stages to citrus peel oil vapours was not studied in detail, it appeared that changes in susceptibility (based on LC_{50} values) were not dramatic for either C.maculatus or D.maculatus (Section 5.3). This suggests that application rates aimed at controlling the most conspicuous stages (eg. adults of C.maculatus) would also give effective control of eggs and larval stages (within grains), thereby giving efficient disinfestation.

The usefulness of essential oils as insecticides is likely to extend beyond beetle pests of storage as these oils were found to

be similarly toxic to the american cockroach, P.americana (Section 5.3) and to the fly, Phormia terraenovae (24 h LC_{50} = 4.15 µl/l; K.N. Don-Pedro, unpublished data). Thus, citrus essential oils have the potential for use against public health insects especially as such oils are relatively non-toxic to man. For example, the volatility of essential oils could make them useful ingredients of aerosols for domestic use.

Limepeel oil and other citrus peel oils tested in this work had a fumigant toxicity against insects which appeared comparable with those of commercial synthetic fumigants (Table 6.2). In some cases the data obtained from the literature were not directly comparable due to differences in exposure times, test species, and methods of expressing effective doses. However, if exposure time is ignored, phosphine appears to be the only synthetic fumigant that is active at much lower concentrations than citrus oil vapours (Table 6.2). In addition, the dosages at which phosphine is most frequently used against storage pests (1 - 2 mg/l; Winks, 1973) are comparable with the LC_{50} for limepeel oil vapour toxicity.

Bioassays revealed that the sorption of limepeel oil fumes occurred with grains and dried fish which appeared to be governed primarily by absorption. This suggested that these oils could be retained at a sufficient concentration within a closed system to remain lethal to pest organisms within or outside the commodity. If sorption was governed by chemical reaction or adsorption it would have severely limited bioavailability in the storage system (Section 5.4). Synthetic fumigants (eg. phosphine) are also known to undergo sorption on storage commodity (Busvine, 1971).

Table 6.2 Comparative toxicity of fumigants against stored products insects.

Compound	Effective concentration (mg/l)	LC_{50} (mg/l)	Exposure time (h)	Insect species	Stage treated	Ref.
Carbon bisulphide	NA	92.02	5-7	S.oryzae	Egg	1
	NA	37.36	5-7	T.castaneum	Egg	1
Methyl bromide	NA	5.95	5-7	S.oryzae	Egg	1
				T.castaneum	Egg	1
Methyl bromide	2.0 - 2.5	NA	NA	S.zeamais	Adult	2
Phosphine	0.0011	0.67+	NA	T.castaneum	Adult	3
	0.533	0.28+	NA	T.castaneum	Adult	3
	51.01	5.40+	NA	T.castaneum	Adult	3
	2.2	11.0+	NA	S.granarius	Adult	4
	1.0	6.0+	NA	S.granarius	Adult	5
	0.014	4.5+	NA	Ephestia ellutella	Larva	6
	4.0	19.0+	NA	E.ellutella	Larva	6

Continued.....

Table 6.2 continued.....

Compound	Effective concentration (mg/l)	LC_{50} (mg/l)	Exposure time (h)	Insect species	Stage treated	Ref.
Limepeel oil (vapour)	NA	6.87	24	C.maculatus	Adult	7
	NA	6.68	24	C.maculatus	Egg	7
	NA	7.79	24	C.maculatus	Larva	7
	NA	10.48	24	S.zeamais	Adult	7
	NA	10.99	24	D.maculatus	Adult	7
	NA	16.22	24	P.americana	Adult	7
	NA	3.57	24	P.terraenovae	Adult	7

NA = Not applicable.

1 = Mostafa et al. (1972); 2 = Bell and MIlls (1983); 3 = Winks (1973); 4 = Monro et al. (1961);

5 = Bang and Telford (1966); 6 = Bell (1979); 7 = Present study.

The insecticidal activity of both fixed and essential oils has been shown to depend primarily on a number of their constituents (Sections 4 & 5). While the toxicity of any of the individual components tested was not conspicuously greater than that of the corresponding natural oil, further work is required to determine whether such compounds have other advantages as control agents compared with the whole oil. For example, despite the inconclusive joint action studies carried out with simple mixtures of citrus oil components the possibility of finding mixtures which could synergise their individual fumigant insecticidal activity remains.

Calculations based on a preliminary market survey showed that the cost of protecting grains or dried fish in order to achieve over 95 % control of beetle pests may be relatively high when industrially extracted oils are used as contact toxicants but relatively low when the more volatile oils are used as fumigants (Table 6.3). Thus, while this tentative costing is of limited value it could imply a substantial cost effectiveness for natural oils which are in most cases available far more cheaply on location in tropical villages. Furthermore, the true benefits to the farmer in such a situation could even be greater as the farmer would be able to obtain a considerably higher price for his protected produce which could be stored for longer periods than would have otherwise been possible. Singh et al. (1978) in fact reported a cost benefit ratio of nearly 11 based on the use of groundnut oil (5 ml/Kg) on cowpea in Nigeria, although in practical storage management it is difficult to compute realistic cost benefit ratios (P.S. Tyler, personal communication).

Table 6.3 Relative cost of plant oils and some synthetic organic insecticides used in controlling stored products pests.

Compound	U.K Retail cost (£/Kg a.i.)	Recommended rate+	Substrate /insect*	U.K Cost of pest control (£) per 1000 Kg substrate	U.K Cost of pest control (£) per m³ (chamber)
Fixed oils (eg. groundnut, corn oil)	1	11.06 g/Kg	Wheat/ cowpea	11.0	
		44.24 g/Kg	Dried fish	44.0	
Citruspeel oils for baking‡ (lime-peel/orangepeel)	2	4.93 g/Kg	Cowpea/ wheat	10.0	
		36.65 g/Kg	Dried fish	60.0	
		9.02mg/l	C.maculatus		0.02
		14.32mg/l	S.zeamais		0.03
		19.86mg/l	D.maculatus		0.03
Pirimiphos methyl	113	4.00mg/Kg	Cereals	0.45	
Malathion (2% dust)	37	8.00mg/Kg	Cowpea/ wheat	0.30	
Lindane (1% dust)	42	8.00mg/Kg	Cowpea/ wheat	0.34	

+ >95% Control of F1 progeny development or LC_{95} value against adults.

‡ Citruspeel oils for baking (used in experiments) cost approximately 10x less than brand used in perfumery.

* Retail prices of 1000Kg of cowpea, wheat and dried fish in the U.K. are approximately 1020, 470 and 3000 pounds sterling (£) respectively.

At present, it is mostly in temperate countries with large-scale input-based agriculture that the implementation of pest control packages is dependent on computed cost benefits. For example, in spite of its high cost radiation-sterilization for the eradication of the screw worm, Cochliomyia hominivora (larvae parasitise cattle) in the South-West United States was embarked upon because of an estimated 39:1 to 113:1 cost benefit ratio in its favour (La Chance, 1979). While Pimentel et al. (1981) showed that the huge amount (3.2 billion U.S.$) spent annually on synthetic chemical control in the U.S.A. for crop protection is justified by a cost benefit ratio of 4:1. On the other hand, similar decisions in tropical and subtropical agricultural pest management cannot rely as much on cost benefit analysis for the following reasons: a) the limited availability of data on pest ecology including forecasting, economic injury levels and yield loss determinations (pre-requisites of cost benefit analysis; Ordish, 1952; Norton, 1976).

b) the cost of pest control is borne almost entirely by governments and other institutions (Kumar, 1984) which are usually more interested in sponsoring the cheapest effective control agent available to give effective control levels.

When applied to grains, plant oils were found to stain the testa but this only persisted with the non-volatile fixed oils. However, such stains, which could limit the acceptibility of the white cowpea variety were not visible on wheat or the brown variety of cowpea (more commonly marketed in Nigeria).

In addition, application of fixed and essential oils did not

affect cooking time of cowpea or taste in the present study (see also Singh et al., 1978). However, the essential oils have the slight disadvantage of leaving a characteristic odour on treated materials although the odour disappears within 48 h of exposure to a free air flow.

In preliminary trials, it was found that the germination rate of cowpea seeds fumigated for 3 days with limepeel or orangepeel vapours (98 µl/l) did not significantly differ from controls (K.N. Don-Pedro, unpublished data). Similar observations with fixed oils and cowpeas have been made by Singh et al. (1978).

Overall, the above findings suggest that the peasant farmer in Africa, Asia or Latin America could employ plant oils directly as grain protectants without seriously limiting grain acceptability or damaging their viability after storage. With dried fish, the situation is even more favourable as fixed oils appear to increase the acceptability of this commodity in urban markets in Nigeria (K.N. Don-Pedro, unpublished observations).

No detailed mammalian toxicity tests have been carried out with the fixed and essential oils used in this work. However, both groups of oils are edible (Section 1) and vegetable oils (fixed oils) form the bulk of human oil/fat consumption (Sheppard et al., 1978).

It is concluded that: 1. Fixed and essential oils could be adopted for large scale use by village farmers in developing countries and field experiments are needed to evaluate such

oils further.

2. The use of fixed oils, which are only effective against eggs, is likely to be more restricted than that of citrus peel oils; the latter being active against all life stages of a potentially wide range of insect species.

3. Citrus peel oils are potential sources of highly effective alternatives worldwide to the currently available synthetic organic fumigants. The latter, apart from their high cost, are often highly toxic to man. With further research, cheaper and more efficient methods of oil extraction from citrus peels (which have little or no economic value) and isolation/synthesis of bioactive components may be achieved.

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Appendix 3.1 Two-way ANOVA for data on effect of essential oils on oviposition of C.maculatus (See Table 3.6).

Source of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	36290	36290	37.64	<0.001
OIL	2	36217	18109	18.74	<0.001
DOSE.OIL	2	2521	1261	1.31	>0.05
ERROR	30	28932	964		
TOTAL	35	103961			

Appendix 3.2 Two-way ANOVA: the effects of fixed oils on adult emergence of C.maculatus (see Table 3.5).

Sources of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	24911	24911	119.76	<0.001
OIL	2	90	45	0.22	>0.05
DOSE.OIL	2	96	48	0.23	>0.05
ERROR	30	6242	208		
TOTAL	35	31339			

Appendix 3.3 3-way nested ANOVA: the effects of fixed and essential oils on % adult emergence of C.maculatus (see Table 3.7).

Model	Sources of variation	D.F.	S.S.	M.S.	F-ratio	P
TYPE (of oil; fixed/essential/control)	TYPE	2	15693.74	7846.87	299.96	<0.001
+ TYPE.DOSE	TYPE.DOSE	2	17332.19	8666.10	331.28	<0.001
+ TYPE.DOSE.OIL	TYPE.DOSE.OIL	7	876.02	125.15	4.78	<0.001
	RESIDUAL	59	1543.42	26.16		
	TOTAL	70	35445.38	506.36		

Appendix 3.4 Two-way ANOVA on the effects of fixed oils on adult emergence of S.zeamais (see Table 3.8).

Source of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	21316.0	21316.0	359.46	<0.001
OIL	2	45.4	22.8	0.38	>0.05
DOSE.OIL	2	101.2	50.6	0.85	>0.05
ERROR	30	1779.3	59.3		
TOTAL	35	23242.0			

Appendix 3.5 Two-way ANOVA on the effects of essential oils on emergence of S.zeamais (see Table 3.9)

Source of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	3721	3721	21.50	<0.001
OIL	2	3108	1554	8.98	<0.001
DOSE.OIL	2	4906	2453	14.18	<0.001
ERROR	30	5196	173		
TOTAL	35	16931			

Appendix 3.6 Two-way ANOVA on the effects of fixed oils on D.maculatus larval emergence (see Table 3.10).

Source of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	22151	22151	113.595	<0.001
OIL	2	1598	799	4.097	<0.05
DOSE.OIL	2	410	205	1.051	>0.05
ERROR	30	5838	195		
TOTAL	35	29996			

Appendix 3.7 Two-way ANOVA on the effects of essential oils on emergence of D.maculatus larvae (see Table 3.11).

Source of variation	D.F.	S.S.	M.S.	F-ratio	P
DOSE	1	18090	18090	58.544	<0.001
OIL	2	1856	928	3.003	>0.05
DOSE.OIL	2	352	176	0.570	>0.05
ERROR	30	9270	309		
TOTAL	35	29569			

Appendix 3.8 Effects of fixed and essential oils on percentage adult emergence of D.maculatus: 3-way nested ANOVA (see Table 3.12).

Model	Source of variation	D.F.	S.S.	M.S.	F-ratio	P
+TYPE (FIXED/ESSENTIAL/CONTROL)	TYPE	2	731.59	365.80	4.99	<0.05
+TYPE.DOSE	TYPE.DOSE	2	3003.74	1501.87	18.47	<0.001
+TYPE.DOSE.OIL	TYPE.DOSE.OIL	8	1152.86	144.11	1.772	>0.05
	RESIDUAL	50	4065.34	81.31		
	TOTAL	62	8953.53	144.41		

Appendix 4.1 Eggs laid by C.maculatus on fixed oil-treated and untreated cowpeas (see Fig. 4.1).

	$\bar{x}$ eggs laid per female (Day[+])							
Treatments (ml/Kg)	1 P<0.01	2 P>0.05	3 P>0.05	4 P>0.05	5 P>0.05	6 P>0.05	7 P>0.05	Total mean P>0.05
Control	26.3	18.0	17.2	14.0	10.5	8.5	6.2	100.7
Groundnut oil (3.5)	13.5**	13.3	15.5	10.0	10.7	6.8	6.2	75.8
(14)	15.8**	18.8	14.7	12.7	12.8	8.3	4.5	87.5
Traditional coconut (3.5)	17.7**	16.2	12.8	10.7	9.8	7.0	3.7	77.7
(14)	12.3***	15.8	22.8	15.3	12.8	6.8	5.5	91.5
Pooled S.E.'s	2.5	2.5	2.4	1.8	1.6	1.5	1.5	7.4

[+] n = 6 (1♀ , 1♂ per replicate)

, * Significantly different from control at P<0.01 and P<0.001 respectively.

Appendix 4.2 Effects of fixed oils on daily egg laying of C.maculatus in a two-way choice oviposition experiment (see Fig. 4.2).

Treatments (ml/Kg)	Total eggs laid per day							
	1	2	3	4	5	6	7	Totals
Groundnut(14)	25***	32***	26***	17***	10***	8***	2***	120***
Untreated	78	68	91	66	58	42	26	429
Groundnut(3.5)	42	28***	28***	24***	10***	8***	6*	146***
Untreated	52	60	59	64	48	34	18	335
Trad. coconut oil (14)	22***	5***	4***	5***	0***	2***	1***	39***
Untreated	54	53	60	48	39	25	20	299
Trad. coconut oil (3.5)	16*	17***	21***	15***	11***	6**	4*	90***
Untreated	30	59	65	56	39	21	14	284
CONTROLS+								
Untreated	15	19	19	19	13	6	4	95
Untreated	15	19	17	20	10	8	3	92
Groundnut(14)	9	8	7	7	4	3	3	41
" (14)	13	6	7	11	4	4	4	49
Groundnut(3.5)	7	6	7	6	4	1	2	33
(3.5)	7	5	7	4	2	3	2	30
Trad. coconut oil (14)	17	1	8	6	8	6	2	48
" (14)	14	1	5	6	6	4	3	39
Trad. coconut oil (3.5)	6	11	10	7	7	5	2	48
" (3.5)	10	5	7	9	5	4	2	42

*, **, *** Significantly different by Chi-square from untreated at P<0.05, P<0.01 and P<0.001 respectively.

+ No significant difference (P>0.05) between pairs.

Appendix 4.3 Toxicity of groundnut and traditional coconut oils applied onto cowpea grains against eggs of C.maculatus (see Table 4.8)

Treatments (ml/Kg)	Total No. of eggs laid	Egg mortality	Adult emergence
Control	362	37	279
Groundnut (1.75)	328	89	211
(3.5)	337	149	151
(7.0)	365	221	71
(10.5)	342	319	15
(14.0)	296	294	4
Traditional coconut oil(1.75)	290	56	187
(3.5)	82	31	47
(7.0)	77	39	24
(10.5)	340	284	37
(14.0)	349	328	3

n = 5 (4 insects [2♀, 2♂] per replicate).

Appendix 4.4 Toxicity of some fatty acid components of fixed oils applied as grain treatments against eggs of C.maculatus (see Table 4.9).

Treatments (ml/Kg)	Total			
	Eggs laid	Unhatched eggs (Mortality)	Adult emergence	% adult emergence
Control - acetone treated	455	22	316	69
Lauric acid (1.75)	387	29	252	65
(3.5)	545	46	304	56
(7.0)	302	89	168	56
(10.5)	332	77	184	57
(14.0)	437	115	223	51
Control - untreated	554	92	368	66
Oleic acid (0.88)	310	88	155	50
(1.4)	322	149	119	37
(1.75)	207	150	33	16
(3.5)	318	282	16	5
(7.0)	287	286	0	0
(10.5)	342	342	0	0
(14.0)	503	503	0	0
Control - untreated	554	92	368	66
Linoleic acid(3.5)	558	96	189	34
(7.0)	380	183	86	23
(10.5)	337	270	109	32
(14.0)	434	270	67	15

n = 4 (4 insects [2♀, 2♂] per replicate).

Appendix 4.5 Toxicity of groundnut oil and traditional coconut oil applied by dipping against eggs of C.maculatus (see Table 4.10).

Treatments (%)+	Total eggs	No. dead	Adult emergence
Control acetone-treated	74	14	45
Groundnut oil (3.2)	62	18	30
(6.3)	53	21	28
(12.5)	66	50	14
(25.0)	87	85	0
(50.0)	94	94	0
Trad. coconut oil (3.2)	80	27	48
(6.3)	78	35	35
(12.5)	82	51	26
(25.0)	75	67	4
(50.0)	61	61	0

+ Acetone based solutions.

Appendix 4.6 Toxicity of fixed oil components applied by dipping against eggs of C.maculatus (see Table 4.11).

Treatments (%)[+]	Egg number	Egg Mortality[†]	Adult emergence
Control (acetone treated)	65	12	40
Lauric acid (1.25)	66	19	39
(2.5)	64	27	29
(5.0)	60	43	16
(10.0)	70	58	12
Oleic acid (1.6)	81	24	49
(3.1)	59	31	24
(6.3)	65	51	9
(12.5)	60	56	0
(25.0)	63	63	0
Linoleic acid (0.8)	63	20	36
(1.6)	53	27	24
(3.1)	60	34	28
(6.3)	67	56	11
(12.5)	49	49	0

† No. of unhatched eggs.

+ Acetone based solutions.

Appendix 4.7 Toxicity of groundnut oil applied onto dried fish strips against eggs of D.maculatus (see Table 4.12).

Dosage (ml/Kg)	No. tested	No. responding
0	30	2
14.0	30	8
17.0	25	13
21.0	26	19
28.0	26	21

Appendix 5.1 Toxicity of citrus oils on cowpea grains against C.maculatus adults (24h response, see Table 5.1)

Limepeel oil			Tangerinepeel oil		
Dose (ml/Kg)	No. tested	No. responding	Dose (ml/Kg)	No. tested	No. responding
0.0	60	0	0.0	60	0
0.86	60	1	0.86	60	1
1.75	60	4	1.75	60	3
2.50	45	22	2.50	45	7
3.50	60	39	3.50	60	12
5.0	45	40	5.0	45	27
7.0	60	60	7.0	60	51

Appendix 5.2 Toxicity of citrus oils on wheat grains against S.zeamais adults (24h response, see Table 5.2).

Limepeel oil			Tangerinepeel oil		
Dose (ml/Kg)	No. tested	No. responding	Dose (ml/Kg)	No. tested	No. responding
0.0	60	0	0.0	60	0
2.5	40	3	2.5	40	1
3.5	60	13	3.5	40	8
5.0	40	27	5.0	40	18
6.0	40	38	7.0	60	47
7.0	60	60	10.5	60	60

Appendix 5.3 Toxicity of limepeel on dried fish against adults of D.maculatus (24h response, see Table 5.3).

Limepeel oil		
Dose (ml/Kg)	No. tested	No. responding
0.0	60	0
7.0	30	4
10.5	30	9
14.0	60	30
28.0	60	49
35.0	60	55
42.0	60	60

Appendix 5.4 Toxicity of topically-applied limepeel oil to adult D.maculatus in airtight and open chambers (24h response, see Table 5.4).

Limepeel oil (closed chamber)			Limepeel oil (open chamber)		
Dose (µl/insect)	No. tested	No. responding	Dose (µl/insect)	No. tested	No. responding
0.0	20	0	0.0	20	0
0.25	20	1	1.0	20	0
0.50	20	7	2.0	20	3
1.0	20	15			
1.5	20	20			

Appendix 5.5 Fumigant toxicity of citrus oils against C.maculatus adults (24h response, see Table 5.13).

Treatments	Dosage[+] - Response (n = 30)					
	0.0	6.0	7.0	8.0	9.0	10.0
Limepeel oil	0	3	5	13	21	30
Tangerinepeel oil	0	3	10	15	18	30
Lemonpeel oil	0	2	8	16	23	30
Grapefruitpeel oil	0	1	4	13	18	30
Orangepeel oil	0	2	7	11	17	30
Mandarinpeel oil	0	2	8	10	16	30

[+] µl/l

Appendix 5.6 Fumigant toxicity of citrus oils against S.zeamais adults (24h response, see Table 5.14).

Limepeel oil			Tangerinepeel oil		
Dosage (µl/l)	No. tested	No. responding	Dosage (µl/l)	No. tested	No. responding
0.0	45	0	0.0	45	0
8.0	30	1	8.0	30	1
10.0	60	12	10.0	60	8
12.0	30	18	12.0	30	15
14.0	45	32	14.0	45	33
16.0	45	45	16.0	45	45
Orangepeel oil			**Lemonpeel oil**		
0.0	45	0	0.0	45	0
8.0	30	2	10.0	45	3
10.0	60	16	12.0	30	12
12.0	30	19	14.0	45	33
14.0	45	32	16.0	30	29
16.0	45	45			
Grapefruitpeel oil			**Mandarinpeel oil**		
0.0	45	0	0.0	45	0
10.0	45	2	10.0	45	6
12.0	30	11	12.0	30	15
14.0	45	22	14.0	45	24
16.0	30	27	16.0	30	26

Appendix 5.7 Fumigant toxicity of citrus oils against D.maculatus adults (24h response, see Table 5.14).

Treatments	Dosage[+] - Response (n = 30)					
	0.0	10.0	14.0	16.0	20.0	24.0
Limepeel oil	0	4	22	25	27	30
Tangerinepeel oil	0	11	22	25	26	30
Orangepeel oil	0	8	21	22	26	30
Lemonpeel oil	0	3	7	13	23	-
Grapefruitpeel oil	0	5	10	13	25	-
Mandarinpeel oil	0	4	12	16	24	-

[+] µl/l

Appendix 5.8 Fumigant toxicity of citrus oils to eggs of C.maculatus (24h response, see Table 5.16).

Limepeel oil			Tangerinepeel oil		
Dose (µl/l)	No. tested	No. responding	Dose (µl/l)	No. tested	No. responding
0.0	58	8	0.0	58	8
4.0	31	6	4.0	35	7
6.0	29	11	5.0	42	16
8.0	33	15	6.0	36	22
9.0	29	24	8.0	31	21
10.0	63	62	9.0	38	27
			10.0	53	45
Orangepeel oil			**Grapefruitpeel oil**		
0.0	58	8	0.0	58	8
4.0	58	14	6.0	47	13
5.0	30	12	8.0	37	18
6.0	28	15	9.0	35	28
8.0	30	21	10.0	118	116
10.0	71	59			
Lemonpeel oil			**Mandarinpeel oil**		
0.0	58	8	0.0	58	8
6.0	45	9	6.0	41	15
8.0	29	11	8.0	32	18
9.0	33	24	9.0	30	19
10.0	140	135	10.0	113	101

Appendix 5.9 Fumigant toxicity of citrus oils to D.maculatus eggs (24 or 48h response, see Table 5.17).

Limepeel oil - 24h			Orangepeel oil - 24h		
Dose (µl/l)	No. tested	No. responding	Dose (µl/l)	No. tested	No. responding
0.0	30	3	0.0	30	3
18.0	30	12	20.0	30	14
24.0	30	20	22.0	30	18
28.0	30	22	28.0	30	22
34.0	30	28	34.0	30	26
Limepeel oil - 48h			**Orangepeel oil - 48h**		
0.0	30	0	0.0	30	5
14.0	30	13	14.0	30	14
16.0	30	20	16.0	30	22
20.0	30	23	20.0	30	26

Appendix 5.10 Fumigant toxicity of citrus oils to early larvae and late stage larvae/pupae of C.maculatus (24h response, see Table 5.18).

Limepeel oil - early larvae			Orangepeel oil - early larvae		
Dose (µl/l)	No. tested	No. responding	Dose (µl/l)	No. tested	No. responding
0.0	44	3	0.0	44	3
6.0	41	9	6.0	34	4
8.0	44	19	8.0	33	14
10.0	42	26	12.0	29	22
12.0	41	32	16.0	70	65
20.0	41	39	20.0	40	40
Tangerinepeel oil - early larvae			**Limepeel oil - late larvae/pupae**		
0.0	44	3	0.0	24	1
6.0	31	3	8.0	29	3
12.0	45	33	16.0	72	36
16.0	48	44	28.0	28	20
20.0	43	42	32.0	27	22
Orangepeel oil - late larvae/pupae			**Tangerinepeel oil - late larvae/pupae**		
0.0	24	1	0.0	24	1
8.0	28	5	8.0	30	10
12.0	24	13	12.0	24	11
16.0	82	60	16.0	77	55
22.0	23	19	22.0	34	27
28.0	27	27	32.0	24	22

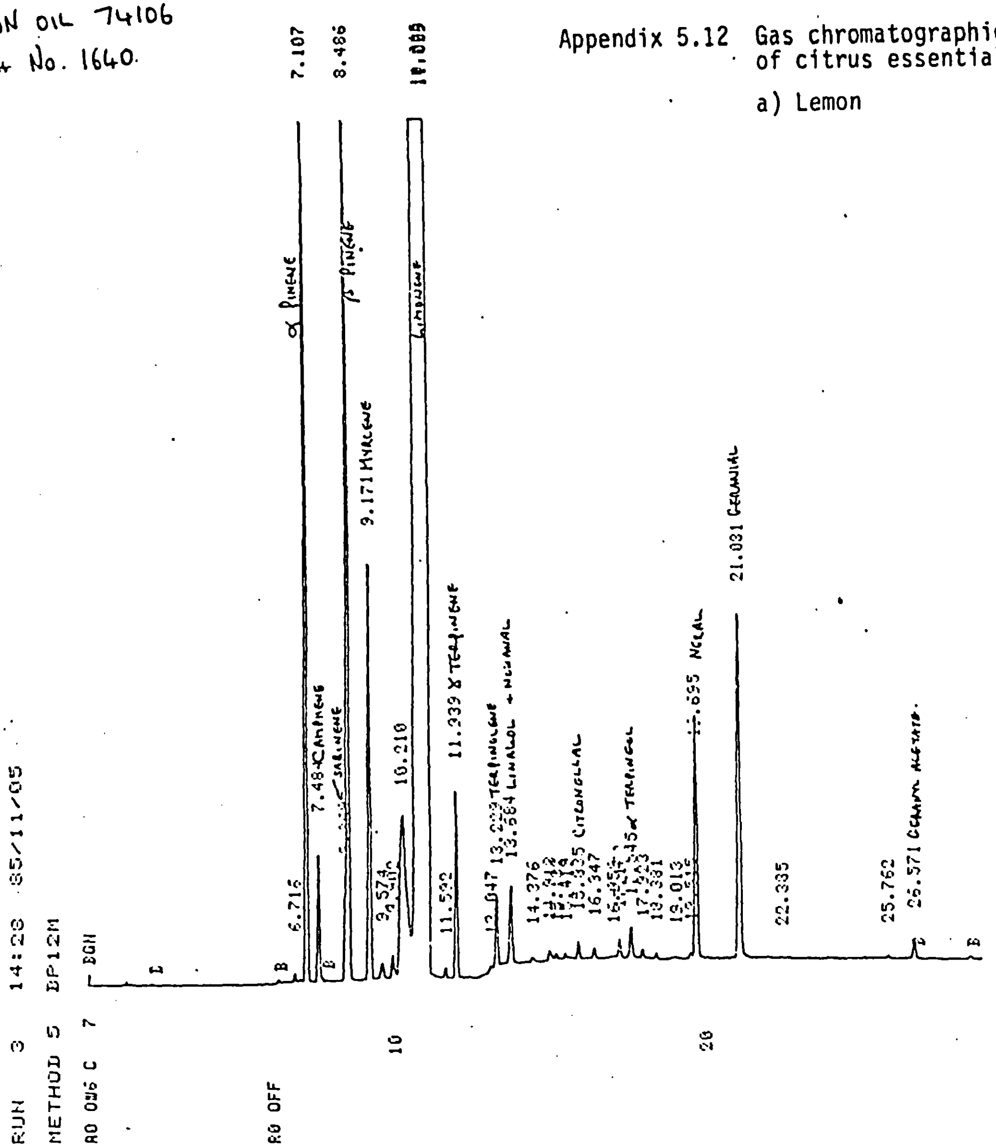
Appendix 5.11 Fumigant toxicity of citrus oils to late larvae and pupae of D.maculatus (48h response, see Table 5.19).

Limepeel oil - late larvae			Orangepeel oil - late larvae		
Dose (µl/l)	No. tested	No. responding	Dose (µl/l)	No. tested	No. responding
0.0	30	0	0.0	30	0
12.0	30	3	12.0	30	7
16.0	30	11	16.0	30	17
24.0	30	15	24.0	30	22
28.0	30	17	28.0	30	24
32.0	30	22			
Limepeel oil - pupae			Orangepeel oil - pupae		
0.0	30	0	0.0	30	0
16.0	30	5	16.0	30	9
28.0	30	20	20.0	30	18
34.0	30	23	28.0	30	22
36.0	30	25	34.0	30	25
40.0	30	27	40.0	30	26

LEMON OIL 74106
BATCH No. 1640.

Appendix 5.12 Gas chromatographic analysis of citrus essential oils.

a) Lemon



RUN 3 14:28 85/11/05

METHOD 5 BP12M CALCULATION

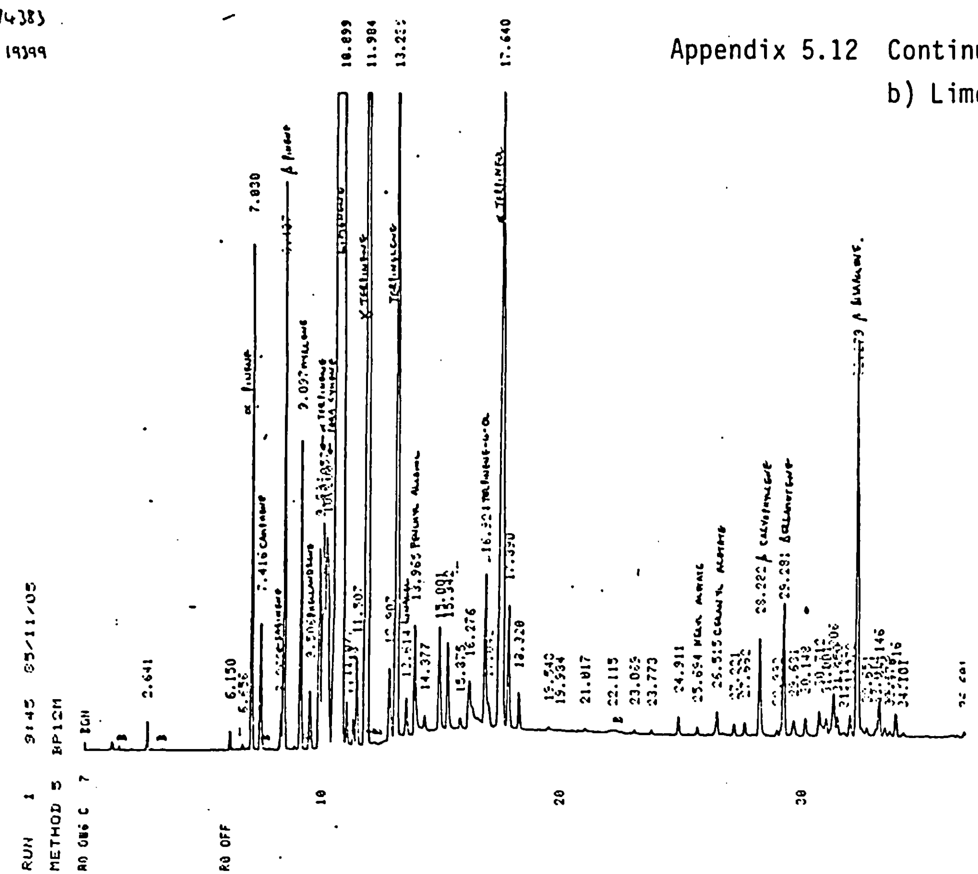
RT	AREA	BC	AREA %	
6.716	0.1117	T	0.0288	
7.107	12.7729	V	3.2985	
7.484	1.4954		0.3862	
8.363	0.9057	T	0.2339	
8.486	13.5023	T	3.4879	
9.171	5.4329	T	1.4031	
9.574	0.4276	T	0.1104	
9.802	0.4511	T	0.1165	
10.210	6.8200	T	1.7614	
10.335	166.7132	T	43.0555	Limonene
10.988	55.9337	T	14.4456	
11.003	12.5322	T	3.2375	
11.029	31.0384	T	8.0173	
11.059	46.6113	T	12.0402	
11.392	0.1296	TS	0.0334	
11.939	2.0635	T	0.5325	
13.047	0.2046	T	0.0528	
13.229	1.3141	T	0.3394	
13.684	3.7414	T	0.9663	
14.376	0.1274	TS	0.0329	
14.918	0.6466	T	0.1670	
15.132	0.6902	T	0.1783	
15.419	0.9152	T	0.2364	
15.835	1.1330	T	0.2926	
16.347	1.3114	T	0.3387	
16.955	0.5264	T	0.1359	
17.152	0.8626	T	0.2228	
17.545	1.3632	T	0.3521	
17.923	0.7709	T	0.1991	
18.381	0.7504	T	0.1938	
19.013	0.6725	T	0.1737	
19.515	0.5156	T	0.1332	
19.695	4.9140	T	1.2694	
21.031	[illegible]	-	[illegible]	
22.385	0.1098	TS	0.0283	
25.762	0.1006	V	0.0259	
26.571	0.2633		0.0680	
47.827	0.1016	H	0.0262	
49.047	0.3966	T	0.1025	
53.054	0.1231	T	0.0319	
53.373	0.0946	T	0.0244	
54.224	0.2174	T	0.0561	
54.507	0.1552	T	0.0400	
54.649	0.1236		0.0319	

60 PEAKS > AREA/HT REJECT

LIME OIL 74383
BATCH No. 19399

Appendix 5.12 Continued......

b) Lime



RUN 1 9145 85/11/05

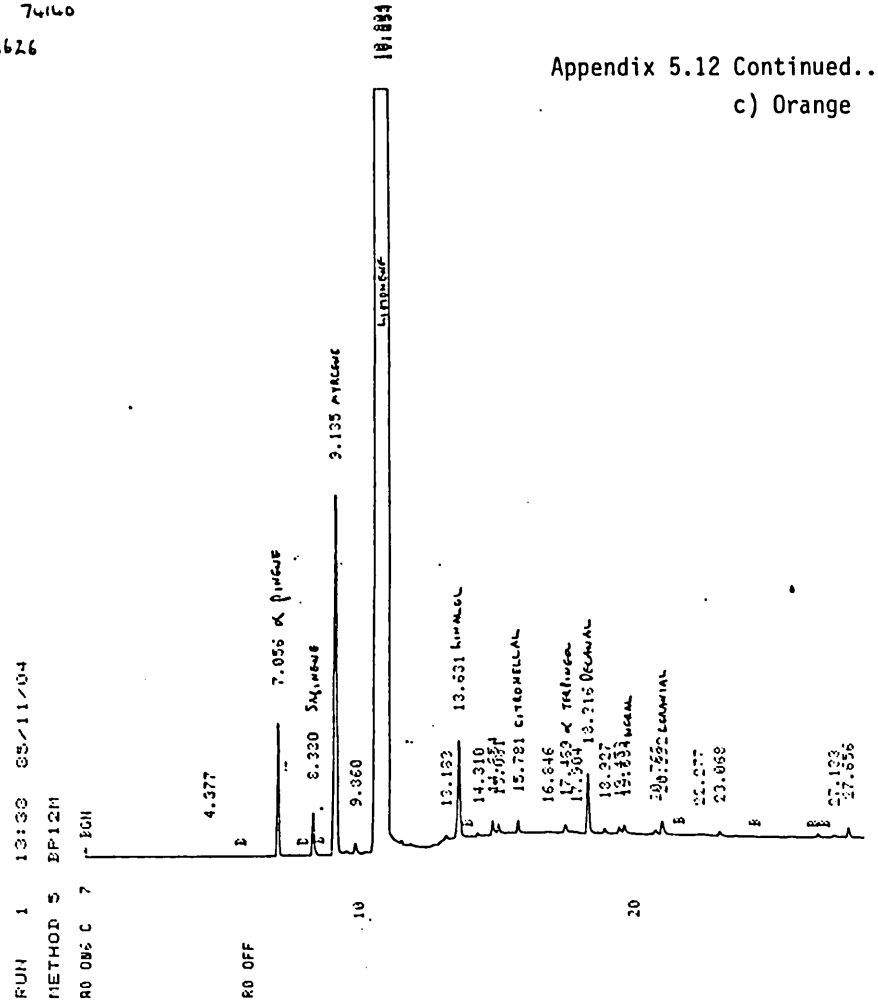
METHOD 5 BP12M CALCULATION: %

RT	AREA	BC	AREA %
2.641	0.3309	T	0.0840
6.150	0.2231	T	0.0567
6.596	0.0981	T	0.0249
7.030	6.5173	U	1.6553
7.415	2.2033		0.5597
8.262	0.5700	T	0.1448
8.407	9.2273	T	2.3423
9.037	4.9230		1.2507
9.506	1.1934	U	0.3032
9.834	4.2775	T	1.0867
10.057	5.2544	T	1.3349
10.200	7.3470	T	1.8665
10.899	211.6106	T	53.7610
11.077	0.5723	TS	0.1454
11.349	0.4044	TS	0.1027
11.507	1.4163	US	0.3598
11.984	40.8963		10.3901
12.907	1.6467	T	0.4183
13.235	20.3350	T	5.1673
13.614	[illegible]	TS	0.1904
13.965	3.1795	T	0.9047
14.377	1.5300	T	0.4019
15.001	3.1829	T	0.8101
15.342	2.9526	T	0.7419
15.675	0.9929	T	0.2523
16.276	3.9613	T	1.0069
16.324	4.7375	T	1.2133
17.042	0.1209	TS	0.0305
17.640	22.4380	T	5.7006
17.850	3.0544	T	0.7760
18.319	3.6647	T	0.9310
19.549	0.1624	TS	0.0412
19.934	0.0863	TS	0.0219
21.017	0.1330	TS	0.0145
22.115	0.1339		0.0340
23.069	0.0945	T	0.0240
23.773	0.1102	T	0.0280
24.911	0.4135	T	0.1050
25.694	0.2179	T	0.0553
26.515	0.6370	T	0.1618
27.221	0.2521	U	0.0640
27.562	0.2635	T	0.0669
28.222	2.1513	T	0.5465
28.892	0.1209	T	0.0307
29.231	2.7613	T	0.7015
29.601	0.5063	T	0.1286
30.148	0.4678	T	0.1189
30.712	0.8407	T	0.2135
31.001	0.4587	T	0.1163
31.306	1.2290	T	0.3122
31.450	0.4553	T	0.1159
31.712	0.1636	T	0.0430
31.971	0.4790	T	0.1217
32.273	8.7078	T	2.2122
32.651	0.1167	TS	0.0296
33.014	0.1259	TS	0.0319
33.146	0.7599	TS	0.1930
33.373	0.1810	TS	0.0460
33.576	0.1162	TS	0.0295
33.816	0.4855	TS	0.1233
34.101	0.1011	TS	0.0255
35.601	0.1524	US	0.0387
38.776	0.1622	U	0.0412
40.926	0.0849	T	0.0215

61 PEAKS > AREA/HT REJECT

ORANGE OIL 74160
BATCH NO: 16626

Appendix 5.12 Continued.....
c) Orange



RUN 1 10:38 85/11/04

METHOD 5 BP12M CALCULATION: %

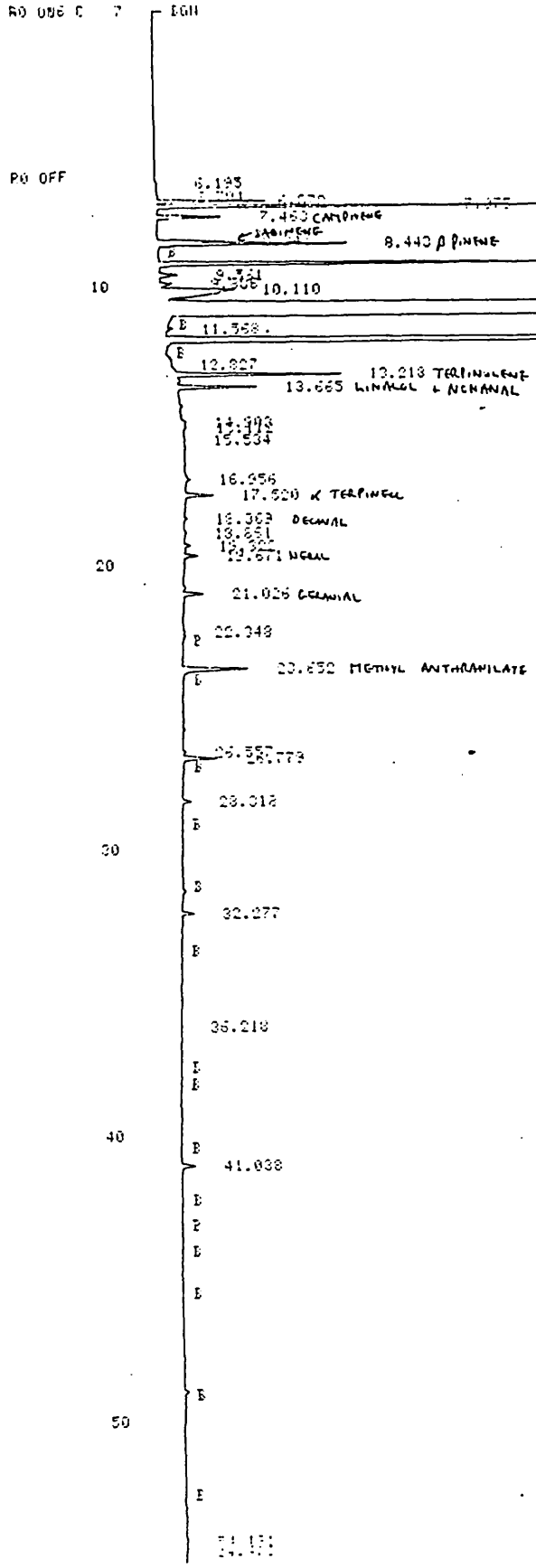
RT	AREA	BC	AREA %	
4.377	0.1043	B	0.0059	
7.056	1.7421	T	0.4340	
8.330	0.6996		0.1742	
9.135	7.3412	T	1.8280	
9.360	0.2172	BS	0.0540	
10.884	101.1781	T	25.1943	
10.945	128.9359	T	32.1200	Limonene
10.999	30.0197	T	7.4751	
11.054	122.8707	T	30.5957	
13.183	0.2575	T	0.0641	
13.631	1.9400		0.4830	
14.310	0.0909	V	0.0226	
14.854	0.2727	T	0.0679	
15.081	0.2426	T	0.0606	
15.781	0.3459	V	0.0856	
16.646	0.1102	T	0.0274	
17.469	0.2696	T	0.0671	
17.804	0.0866	V	0.0216	
18.315	1.1377	T	0.2957	
18.527	0.1264	TS	0.0314	
19.453	0.1912	T	0.0476	
19.634	0.3259	T	0.0811	
20.763	0.1063	T	0.0264	
20.952	0.0245	V	0.0060	
23.277	0.1206	T	0.0300	
23.068	0.1298	V	0.0323	
27.133	0.0810	V	0.0201	
27.856	0.2040	T	0.0508	
30.720	0.1388	V	0.0345	
31.672	0.1105	V	0.0275	
32.618	0.0994	T	0.0247	
33.392	0.0904	V	0.0225	
39.035	0.0872	T	0.0217	
49.704	0.1819	T	0.0453	

55 PEAKS > AREA/HT REJECT

Mandarin oil 74221
Batch No 17625.

Appendix 5.12 Continued.....

d) Mandarin



RUN 2 11:55 85/11/05

METHOD 5 EP12M CALCULATION: %

RT	AREA	BC	AREA %	
6.195	0.0880	T	0.0220	
6.704	0.1421	T	0.0356	
6.978	1.3230	T	0.3330	
7.075	4.9726	T	1.2469	
7.463	1.0147	T	0.2544	
8.345	0.8489	T	0.2128	
8.443	2.5910		0.6497	
9.155	6.1898	T	1.5522	
9.561	0.3739	VS	0.0937	
9.986	0.2387	T	0.0598	
10.110	3.5130	T	0.8809	
10.960	224.2506	T	56.2356	
10.971	0.4747	TS	0.1190	Limonene
10.989	16.8912	T	4.2357	
11.058	93.4653		23.4381	
11.568	0.0670	V	0.0218	
12.027	30.0689	T	7.5403	
12.827	0.2222	T	0.0557	
13.218	3.0073	T	0.7541	
13.665	2.0660	T	0.5180	
14.203	0.0926	TS	0.0232	
15.112	0.1245	TS	0.0312	
15.533	0.0863	VS	0.0216	
16.956	0.2945	T	0.0738	
17.520	0.6860	T	0.1720	
18.369	0.1095	T	0.0274	
18.861	0.1133	T	0.0284	
19.322	0.1380	T	0.0346	
19.670	0.3632	T	0.0910	
21.026	0.4241	T	0.1068	
22.348	0.0968	T	0.0242	
23.652	1.3068		0.3277	
26.557	0.0876	T	0.0219	
26.779	0.6247		0.1565	
28.318	0.1859	V	0.0466	
32.277	0.2232	T	0.0559	
36.218	0.0948	T	0.0237	
41.038	0.2996	V	0.0751	
54.191	0.0912	T	0.0228	
54.473	0.1379	V	0.0345	

60 PEAKS ; AREA%NT REJECT

Appendix 5.13 Fumigant toxicity of liquid citrus oil components against C.maculatus adults (24h response, see Table 5.20).

Treatments	Dosage response[+] (n = 30)																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	20	0.0
D-limonene					2	7	9	16	30								0
Citronellal			6	-	15	21	29	-	30								0
n-decyl-alcohol									0							0	0
n-tridecane									2	6	11	-	18				0
Nonane									0							0	0
n-undecane									11	20	25	0	30				0
∝-pinene							1	6	13	19	28						0
∝ -terpinene						7	10	24	30								0
β -myrcene							1	9	19	26	30						0
Citral	1	-	15	18	24	-	30										0
6-methyl-5-hepten-2-one		3	6	22	24	30											0
ɣ -terpinene				11	17	22	27	30									0
Geraniol									2	7	10	-	18	-	26		0
n-decyl aldehyde									0							0	0
Linalool			2	11	26	29	30										0
Toluene									0							0	0
Acetone									0							0	0
Undecanal							2		6	10	15						0
Beta-pinene						3	14	17	28								0
Terpinolene				9	11	-	18	28	30							0	
Myrcene							3	14	20	23	30					0	
Nonanol									0							0	
9-decenal					7		15	28	30							0	
Methyl anthranilate			5	11	22	26	30									0	
∝ -terpineol		9	13	17	28	30										0	

[+] µl/l

Appendix 5.14 Fumigant toxicity of solid citrus oil components against C.maculatus adults (24 and 48h responses, see Table 5.21).

Treatments	Dosage[+] response (n = 30)					
	24h			48h		
	4	6	10	4	6	10
Camphene	0	3	4	2	8	16
Fenchyl alcohol	23	30	30	30	30	30
Capriylic acid	0	0	0	0	0	0
Thymol	0	0	0	0	0	0
(-)Borneol	-	9	4	-	29	30

[+] mg/l

Control mortality = 0

Appendix 5.15 Relative fumigant toxicity of d-limonene against test insect (24h response, see Table 5.22).

Treatments	Dosage+ response (n = 30)										
	6	7	8	9	10	14	16	18	20	22	0
C.maculatus											
D-limonene	2	7	9	16	30						0
S.zeamais											
D-limonene						8	14	18	26	30	0
D.maculatus											
D-limonene					1	9	21	-	24		0

+ µl/l

Appendix 5.16 Relative fumigant toxicity of limepeel oil against test insects (24h response, see Tables 5.13, 5.14, 5.15).

Treatments	24h mortality (95% C.L.)					
	LC_{50} (µl/l)	LC_{95} (µl/l)	Slope±S.E.++	X^2	D.F.	T.F.+
C.maculatus						
Limepeel oil	7.99(7.66-8.32)	10.49(9.81-11.70)	13.88±1.9	0.68	1	1
S.zeamais						
Limepeel oil	12.19(10.73-13.83)	16.66(14.66-20.27)	12.13±2.81	4.31	1	0.66
D.maculatus						
Limepeel oil	12.72(11.63-13.69)	19.60(17.20-22.90)	8.76±1.30	1.60	1	0.63

+ Toxicity Factor (reference - limepeel oil against C.maculatus

++ Data do not contradict the hypothesis of parallelism.

Appendix 5.17 Relative fumigant toxicity of artificial and natural limepeel oil to C.maculatus adults (see Table 5.23).

Treatment	24h Dosage+-Response(n=30)				
	6	7	8	9	10
Artificial lime-peel oil mixture		2	7	18	27
Natural limepeel oil	1	5	13	22	29

+ µl/l

Appendix 5.18 Fumigant toxicity of D-limonene and ∝-terpineol alone, and in binary mixtures to C.maculatus adults (see Fig. 5.1).

Treatments	24h mortality (95% C.L.) LC_{50} (µl/l)	LC_{95} (µl/l)	Slope±S.E.	X^2	D.F.
D-limonene	8.28(7.93-8.68)	11.15(10.30-12.73)	12.74±1.85	3.07	1
∝-terpineol	4.05(3.56-4.48)	8.06(6.60-12.62)	5.50±1.14	5.67	2
D-limonene: ∝-terpineol					
4:1	8.17(7.57-8.83)	13.08(10.46-16.32)	8.08±1.5	3.80	3
3:2	6.45(6.05-6.83)	9.67(8.78-11.42)	9.36±1.4	0.56	1
1:1	4.88(4.55-5.29)	7.53(6.60-9.67)	8.73±1.49	0.60	1
2:3	3.99(3.66-4.29)	6.25(5.58-7.62)	8.42±1.33	2.17	2
1:4	3.54(3.16-3.94)	6.58(5.63-8.47)	6.11±0.86	0.17	2

Appendix 5.19 Joint fumigant toxicity of D-limonene and ∝-terpineol to C.maculatus; data analysed by Hewlett and Plackett Model (see Section 2.2.4, Appendix 5.20).

Dose (µl/l)	No. tested	No. responding	No. expected	X^2
Limonene				
7.0	30	7	4.468	1.685
8.0	30	9	12.498	1.678
9.0	30	16	20.974	3.920
10.0	30	30	26.412	4.075
Terpineol				
3.0	30	6	6.97	0.177
4.0	30	13	16.05	1.245
5.0	30	17	22.95	6.570
6.0	30	28	26.78	0.515
7.0	30	30	28.61	1.462
1:1 mixture				
1 2				
1.5 1.5	30	2	0.86	1.548
2.0 2.0	30	4	5.17	0.321
2.5 2.5	30	17	12.76	2.448
3.0 3.0	30	24	20.12	2.270

Total Chi-square = 27.914; D.F. = 9.0; P<0.001; Number of iterations = 4.0.

Regression equation for Limonene is Y = -13.142 + 14.319x

Regression equation for Terpineol is Y = -3.857 + 6.552x

Appendix 5.20 Fumigant toxicity of D-limonene and ∝-terpineol alone, and in binary mixtures to C.maculatus adults (see Fig. 5.19, 24h response).

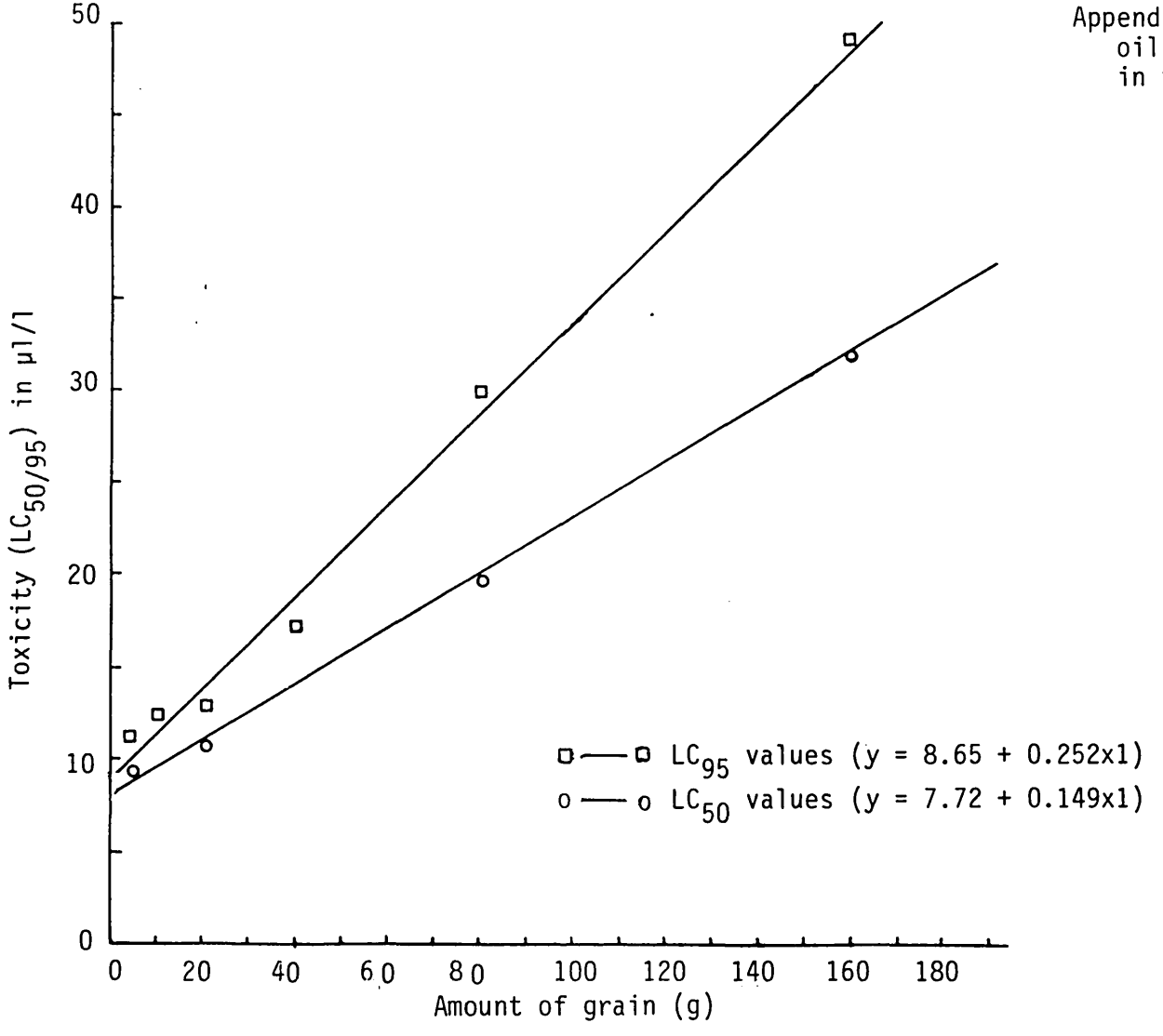
Treatments	Dosage+ - repsonse (n = 30)									
	2	3	4	5	6	7	8	9	10	0
D-limonene					2	7	9	16	30	0
∝-terpineol		9	13	17	28					
D-limonene: ∝-terpineol										
4:1				2	5	6	12	22	30	
3:2				6	10	19	21	30		
1:1		2	4	17	24					
2:3		3	18	24	27					
1:4	2	10	-	24	28					

+ µl/l

Appendix 5.22 Fumigant toxicity of limepeel oil against C.maculatus in the presence of varying amounts of cowpea grains (24h response, see Table 5.25).

Treatment (Amount of cowpea)	24h Dosage+ - Response (n = 30)																
	8	9	10	11	12	13	14	18	20	22	26	28	30	34	38	44	0
Limepeel oil																	
(5g)	3	5	23	28	30												0
(10g)		4	20	23	27	-	30										
(20g)		1	12	-	25	29	30										0
(40g)			7	12	19	20	25										0
(80g)							5	9	12	19	29						0
(160g)										3	8		11	15	20	30	0

+ µl/l



Appendix 5.21 Toxicity of limepeel oil vapour against C.maculatus in the presence of cowpea grain.

Appendix 5.23 Fumigant toxicity of limepeel oil against D.maculatus adults in the presence of dried fish (24h response, see Table 5.26).

Limepeel oil - 10g dried fish

Dose (µl/l)	No. tested	No. responding
0	30	0
150	30	7
250	30	13
300	30	15
350	30	24
400	30	26
450	30	28

Appendix 5.24 Mortality of C.maculatus exposed to limepeel oil vapour in the presence of varying amounts of cowpea grain (see Fig. 5.1).

Treatment (Amount of grain)		Mortality (n = 30) 24h	48h	72h	96h
Control	(0)	0	0	1	2
Control	(160g)	0	0	1	3
Limepeel oil at 10µl/l	(5g)	23	29	30	30
	(10g)	20	28	30	30
	(20g)	12	15	26	30
	(40g)	7	8	16	17
	(80g)	0	1	2	8
	(160g)	0	0	1	5

Appendix 5.25 Speed of fumigant action of limepeel oil and D-limonene against C.maculatus adults.

Limepeel oil - 6µl/l		
Time (h)	No. tested	No. responding
24	30	3
48	30	5
90	30	8
120	30	10
168	30	18
Control(168h)	30	0

Limepeel oil - 8µl/l		
Time (h)	No. tested	No. responding
18	30	2
24	30	5
30	30	8
42	30	14
48	30	23
90	30	28
Control(90h)	30	0

Limepeel oil - 9µl/l		
6	30	3
12	30	7
18	30	11
24	30	14
30	30	17
48	30	21
Control(48h)	30	0

Limepeel oil - 10µl/l		
0.5	30	1
2.0	30	3
5.0	30	8
7.0	30	11
9.0	30	17
12.0	30	21
15.0	30	27
Control(15h)	30	0

Limepeel oil - 14µl/l		
1.0	30	2
2.0	30	5
3.0	30	11
5.0	30	17
7.0	30	25

D-limonene - 6µl/l		
30	30	1
48	30	3
120	30	5
168	30	18
Control(168h)	30	2

Continued overleaf.....

Appendix 5.25 continued.....

Limepeel oil - 14µl/l		
Time (h)	No. tested	No. responding
9.0	30	28
Control(9h)	30	0

D-limonene - 6µl/l		
Time (h)	No. tested	No. responding

D-limonene - 8µl/l		
18	30	2
24	30	3
30	30	5
42	30	17
48	30	21
90	30	29
Control(90h)	30	1

D-limonene - 9µl/l		
12	30	3
24	30	7
30	30	12
48	30	19
72	30	27
Control(72h)	30	0

D-limonene - 10µl/l		
0.5	30	2
2.0	30	7
4.0	30	11
5.0	30	13
7.0	30	15
9.0	30	24
12.0	30	29
Control(12h)	30	0

D-limonene - 14µl/l		
1.0	30	3
2.0	30	8
3.0	30	16
4.0	30	18
5.0	30	21
7.0	30	29
Control(7h)	30	0

Appendix 5.26 Speed of fumigant toxicity of limepeel oil and D-limonene against D.maculatus adults (see Table 5.28).

Limepeel oil - 10µl/l		
Time (h)	No. tested	No. responding
12	30	3
48	30	9
72	30	11
120	30	17
Control(120h)	30	0

Limepeel oil - 16µl/l		
Time (h)	No. tested	No. responding
3	30	5
6	30	10
9	30	15
20	30	18
30	30	23
48	30	26
Control(48h)	30	0

Limepeel oil - 22µl/l		
3	30	7
6	30	16
9	30	17
12	30	20
30	30	26
Control(30h)	30	0

Limepeel oil - 24µl/l		
4	30	1
6	30	3
10	30	7
12	30	10
15	30	17
20	30	23
Control(20h)	30	0

Limepeel oil - 28µl/l		
4	30	1
6	30	3
10	30	7
12	30	15
15	30	19
20	30	28
Control(20h)	30	0

D-limonene - 12µl/l		
24	30	2
32	30	7
48	30	14
72	30	15
96	30	19
Control(96h)	30	0

Appendix 5.26 continued.....

D-limonene - 16µl/l		
Time (h)	No. tested	No. responding
3	30	7
9	30	12
20	30	14
30	30	18
48	30	25
Control(48h)	30	0

D-limonene - 22µl/l		
Time (h)	No. tested	No. responding
3	30	6
6	30	8
9	30	13
20	30	18
30	30	21
Control(30h)	30	0

D-limonene - 24µl/l		
4	30	1
8	30	4
10	30	7
12	30	13
15	30	18
20	30	23
Control(20h)	30	0

D-limonene - 28µl/l		
4	30	2
8	30	7
10	30	12
12	30	17
15	30	22
20	30	26
Control(20h)	30	0

Appendix 5.27 Fumigant toxicity of limepeel oil to cockroaches (24h response, see Table 5.30).

Limepeel oil Dose (µl/l)	No. tested	No. responding
0	15	0
16	10	4
20	15	8
24	15	11
36	15	15

Appendix 5.28 Percentage mortality of C.maculatus adults fumigated with limepeel or limonene oils at 8µl/l over 24 and 48h.

Experiments[+]	% Mortality			
	Limepeel oil		Limonene	
	24h	48h	24h	48h
1	50	60	30	53
2	52	83	23	70
3	20	73	20	70
4	17	77	23	63
5	43	85	14	67
6	67	77	-	

[+] Data pooled from different experiments (n = 30-60).